DEVELOPMENT OF INDIVIDUAL-SPECIFIC RISK ASSESSMENT FOR GLAUCOMA

by

LEUNG, Ka Kit

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LEUNG, Ka Kit

August 29, 2014
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by

LEUNG, Ka Kit

This is to certify that I have examined the above PhD thesis

and have found that it is complete and satisfactory in all respects,

and that any and all revisions required by

the thesis examination committee have been made.

Prof. David CC LAM, Thesis Supervisor

Prof. Qing-Ping SUN, Acting Head of Department

Department of Mechanical and Aerospace Engineering

August 29, 2014
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Abstract

Tonometry is the primary risk assessment method for glaucoma, the leading cause of blindness in Hong Kong. The intraocular pressure (IOP) measured in tonometry is generally compared to a threshold IOP level (21 mmHg) as a reference for clinicians to determine if the subject is at risk. Both the measured IOP and the threshold IOP, however, ignore the individual variations of the ocular tissue biomechanical properties in human eyes. Some studies from the literature have reported increased ocular rigidity in glaucoma patients, and the error in IOP measurement caused by the individual variations in the corneal stiffness can be up to 15 mmHg. These make the existing tonometry fail to accurately distinguish the eyes that are at risk. The inclusion of individual ocular biomechanical properties is needed to improve the accuracy of the measured IOP and the clinical relevance of the threshold IOP, but a non-invasive method for characterizing ocular tissues in clinics is currently unavailable.

In clinics, Goldmann Applanation Tonometry (GAT) is generally regarded as the gold standard of IOP measurement, in which the subject’s cornea is applanated to a specific area (7.35 mm²) and the applied load is acquired to calculate the IOP. As this is a single-point load measurement, the individual corneal stiffness cannot be accounted for in GAT, causing a potential error of up to 15 mmHg. In this thesis, an Individual-Specific Tonometry (IST) is developed to measure the corneal stiffness and the IOP simultaneously. The cornea is indented by a flat-punch indenter in IST, and the load-displacement behavior during the indentation is acquired for the IOP measurement. The method and IOP analysis were verified on porcine eyes ex vivo, in which the IOP of the eyes were controlled by a manometer. The analysis showed that the IOP measurement errors can be more than 50% without individual corneal property correction, but were significantly reduced to less than 10% by incorporating the individual corneal properties into the IOP measurement.

The IOP measured by the tonometry is then compared to a threshold IOP reference to determine if a subject has high risk for glaucoma, while the individual ocular biomechanical properties of the subject are normally ignored during the comparison. 21 mmHg is generally used as the universal reference to distinguish low IOP and high IOP. Clinics has, however, found that eyes with IOP < 21 mmHg may still develop glaucoma, meaning that a universal threshold IOP is not sufficient. In this thesis, a finite element analysis (FEA) model of the eye is developed to determine if the ocular stiffness would affect the threshold IOP. The results showed that optic nerve damage and peripheral vision loss behavior in glaucoma can be phenomenologically modelled by a shear-based damage criterion. Inherently stiffer eyes or eyes with age-stiffened tissues were found to tolerate lower IOP level before the optic nerve being damaged. This means that the threshold IOP reference in tonometry may have to be adjusted individually according to the subject’s ocular tissue stiffness.

Although the measurement of ocular stiffness can be important in the risk assessment, non-invasive way to characterize in vivo ocular tissue properties is currently unavailable. An
instrumented indentation technique is therefore developed for clinical measurement. The method was first verified and benchmarked with the standard 3-point bending method with a silicone eye model. It was then tested on *ex vivo* porcine eyes and human cadaver eyes, in which the IOP of the eyes were controlled by a manometer. The results showed that the stiffness and the tangent modulus of the eyes can be successfully measured by the indentation method. The method was then used to measure the ocular biomechanical properties of the glaucoma subjects and normal subjects in clinics. It was found that the results measured by the indentation method were comparable to the results measured by invasive methods reported in the literature. The indentation method may therefore act as a useful tool to study the effect of ocular stiffness on the threshold IOP in clinics.

With the individual-specific tonometry and the ocular stiffness characterization method, the accuracy of the IOP measurement and the clinical relevance of the threshold IOP in tonometry could be improved in the future. People who are more susceptible to glaucoma may be identified more accurately. This can enable earlier treatment and help arrest vision loss.
Chapter 1

1 General introduction

1.1 Brief introduction to the eye and common eye diseases

1.1.1 Human eye anatomy

Eyes are the organs that receive visual signals and permit vision. Visual light is received by the eyes and converted to electro-chemical impulses. The visual signal is then transferred to the brain for analysis.

A schematic diagram of a human eye is shown in Figure 1-1. Light first passes through the cornea, the anterior chamber (which is filled with aqueous humor to maintain the intraocular pressure (IOP) that retains the spherical shape of the eye), the pupil (which controls the amount of light going into the eyes and protects the eyes from being damaged by high intensity of light. The size of the pupil is controlled by the iris.), the posterior chamber, the lens, the vitreous chamber (which contains vitreous humor), and finally the retina (which lying on the surface of the choroid), which contains photosensitive ganglion cells to receive visual signals. After converting the visual signals into electro-chemical impulses, they are transferred from the retina to the optic disc, passing through the lamina cribrosa (LC) (which is a special portion of the posterior sclera that allows the nerve bundles to pass through), and are finally transferred to the brain for interpretation.

Other than the parts of the eye where light and visual signals pass through, the eye contains other structures to maintain its functions. The retinal blood vessels transport nutrients to the eye. The ciliary muscle of the eye is a circumferential tissue connecting the zonular fibers (which connect the lens with the ciliary muscle) and the sclera, and is responsible for the accommodation (i.e. varying the refractive power of the lens by the contraction of the
ciliary muscle for focusing the image onto the retina). The ciliary body is responsible in aqueous humor production in the posterior chamber for retaining the shape of the eyeball. The sclera is the white part of the eye that contains collagen and elastic fibers and acts as a protective outer layer and is a crucial structural component of the eye. The corneal limbus is the boundary between the cornea and the sclera.

1.1.1.1 Sclera

The sclera is the white and opaque outer layer which maintains the shape of the eye and is made of collagen and elastic fibers [1, 2]. It is approximately spherical in shape for a human eye, with an average diameter of 24 mm [2]. From the anatomy point of view, the human sclera is built of different layers, namely the Tenon’s capsule, the episclera, the stroma and the lamina fusca, where the choroid is attached to it [2].

The main function of the sclera is to act as a protective layer of the eye, and provide resistance against internal and external loading. It also helps the eye to achieve optical stability by being sufficiently rigid to maintain the shape and dimensions of the eye and minimize any ocular deformation.

In addition, there are two special structures in the sclera that help the eye work properly: the trabecular meshwork (TM) and the lamina cribrosa (LC). The trabecular meshwork is a structure where the aqueous humor filters into the Schlemm’s canal and leaves the eye [2]. If the TM is blocked, the intraocular pressure (IOP) in the eye can increase and may lead to eye diseases such as glaucoma; The LC is a small portion of structure in the posterior sclera where the optic nerve exits the eye.

1.1.1.2 Lamina cribrosa (LC)

The lamina cribrosa (LC) is a special portion of the sclera in the optic nerve head (ONH)
region, as illustrated in Figure 1-2. It is the place where the optic nerves pass through and send out vision signals from the retina to the brain for interpretation. Structural abnormalities in the LC are generally believed to be a cause of glaucomatous nerve damage and cause the collapse of the optic disc framework in ONH [2]. It has also been found that high IOP can deflect the LC posteriorly, causing irregular disruption of the optic nerves passing through the LC [3] and damaging the nerves.

1.1.1.3 Intraocular pressure (IOP)

Intraocular pressure (IOP) is the fluid pressure inside the eye and is commonly measured in the unit of mmHg. It is primarily determined by both the production and the drainage of the aqueous humor, which is produced by the ciliary body in the posterior chamber and is drained to the Schlemm’s canal through the TM. When there is any problem in the balance, the aqueous humor in the anterior chamber and the posterior chamber may build up or drain away, causing an abnormality in the IOP.

Normal IOP (generally around 10 - 21 mmHg) is essential to keep the eyeball spherical in shape and to maintain optical stability. High IOP may cause optic nerve damage. It has been found that higher IOP is associated with a higher prevalence of glaucoma (Table 1-1) [4]. It is therefore a common practice for ophthalmologists to measure patients’ IOP and use IOP as a risk assessment parameter for glaucoma.

1.1.2 Eye diseases

There are quite a number of ocular diseases or defects in human eyes. The following provides brief descriptions of some of the most common.

Glaucoma is an eye disease in which the optic nerve is damaged, and can cause irreversible blindness if it is left untreated. It is the second leading cause of blindness in the
world and the leading cause of blindness in Hong Kong. High IOP is currently believed to be the major risk factor for glaucoma, although there are quite a number of glaucoma patients with normal IOP (< 21 mmHg).

Myopia (short-sightedness) and hyperopia (long-sightedness) are two very common optic problems. They are caused by refractive defects of the eyes, and this means that the eye cannot correctly focus light on the retina, and this causes blurring of the vision. Myopia causes the distance vision to appear blurred (Figure 1-3), while hyperopia causes the near vision to be unclear.

A cataract is an eye condition in which the lens becomes cloudy, and partially or completely blocks the passage of the light. The disease often occurs in the elderly and is the leading cause of blindness in the world.

Retinal detachment is a disorder of the eye whereby the retina detaches from the eye. Vision loss and blindness may result if the initial detachment is not treated immediately. Retinal detachment has a higher prevalence in people with a high degree of myopia (> 6 diopters). Approximately 40-50% of retinal detachment patients have myopia [5].

In the literature, the biomechanical properties of the ocular tissues are generally believed to play an important role in different ocular diseases and defects, in particular for glaucoma and myopia. In this chapter, background information and the findings in the literature on glaucoma will be discussed.

1.2 Glaucoma

1.2.1 Characteristics and management of glaucoma

Glaucoma is the term used for a group of eye diseases in which the optic nerve is damaged, and the optic neuropathy can cause visual field loss and blindness [6, 7]. Clinically, vision loss in glaucoma is generally observed to begin at the periphery in the early stage, and
then progresses towards the center [8], as illustrated in Figure 1-4. This disease is often, but not always, associated with a high IOP level (Figure 1-5 and Table 1-1). The optic nerve in the ONH region, in particular in the LC, is believed to be damaged due to the high loading caused by the elevated IOP.

When an eye suffers from glaucoma even when it is at a “normal” IOP level (i.e. < 21 mmHg), it is called normal tension glaucoma (NTG). In contrast, if the eyes do not suffer from glaucoma while the IOP is high (i.e. ≥ 21 mmHg), it is called “ocular hypertension”.

There are two major categories of glaucoma: open-angle glaucoma (OAG) and angle-closure glaucoma (ACG). The more common OAG is a chronic glaucoma in which the vision loss of the patient progresses slowly and it is not easy to recognize at the early stage, while the ACG can be acute without any advance symptoms and can cause discomfort and rapid vision loss [9].

Most of the treatments for glaucoma aim to reduce the patients’ IOP, even for cases of NTG. This can be done by using drugs (e.g. timolol) and surgery (e.g. canaloplasty and trabeculectomy), aiming at reducing aqueous humor production or enhancing aqueous humor drainage. Nonetheless, the vision lost before treatment cannot be recovered even though the progression of the disease can be slowed down or stopped.

1.2.2 Prevalence of glaucoma

Glaucoma is currently the second leading cause of blindness in the world, accounting for 14% of the world blindness [10, 11]. In Hong Kong, glaucoma is the leading cause of blindness (23%) [12]. It was estimated that there will be more than 79 million of people in the world suffering from OAG and ACG by 2020 [13].

The prevalence of glaucoma is found to vary with race and age [13-24], as shown in Figure 1-6 and Figure 1-7. OAG was observed to be more prevalent in Africa, while ACG was
observed to be more prevalent in China [13]. For all ethnicities, glaucoma was found to be much more common in the elderly [13].

1.2.3 Existing intraocular pressure (IOP) measurement methods

IOP measurement is important in both the risk assessment and management of glaucoma. The current measurement methods can typically be classified into 3 types: palpation, manometry and tonometry.

Palpation is the oldest method to estimate IOP, in which the patient is asked to close the eyes in downward gaze and the examiner uses the index finger to sense the IOP on the redundant skin of the upper eyelid. The accuracy of the measurement is doubtful and highly depends on the experience and perceptivity of the examiner.

Manometry is an invasive measurement method where a needle is connected to the eye. Though it is the most accurate method to measure IOP, it is seldom used on humans due to the risk of bleeding and infection.

Tonometry is an instrumented method to measure IOP with minimum disturbance to the eye. The method generally measures the IOP through the cornea on the basis of indentation or applanation. The most frequently used tonometers include: the Goldmann Applanation Tonometer (GAT), the Non-contact Tonometer (NCT) and the Dynamic Contour Tonometer (DCT), as shown in Figure 1-8.

GAT was introduced by Goldmann and Schmidt in 1957 [25]. It is now considered the gold standard in tonometry and is the most commonly used method of measuring IOP in clinics. It measures the IOP by acquiring the force used to flatten the cornea with a predetermined applanation contact area of $7.35\text{mm}^2$ (i.e. a diameter of 3.06mm).

The Non-Contact Tonometer (NCT) uses a rapid air pulse to flatten the cornea. An electro-optical system is used to detect the corneal applanation for IOP estimation. Since NCT
does not require direct contact to the cornea, topical anesthetic eye drops are not needed. Thus NCT is generally considered to be an IOP screening method for children or non-compliant patients.

The Dynamic Contour Tonometer (DCT) is an instrument that uses the principle of corneal contour matching instead of the typical applanation or indentation. It uses a miniature pressure sensor embedded in the tonometer tip, which has a contour that matches the corneal shape, to measure the IOP.

1.2.4 Existing findings in the biomechanics of glaucoma

IOP is commonly regarded as a crucial element in the causes of glaucoma. A high IOP can cause glaucomatous damage to the optic nerves in the LC [3, 13, 26, 27] and IOP is used as the primary risk factor for glaucoma in clinics. From experiments on human cadaver eyes, it was found that elevated IOP can lead to bowing of the LC (Figure 1-9), and the axonal bundles of the optic nerve in the LC are sheared and disrupted under high IOP (Figure 1-10).

From the literature, computation models have been used to understand the biomechanical linkage between IOP and glaucoma [28-31]. Tangential tensile strains in the tissues were correlated with IOP and other structural parameters in the literature [28]. Out of a number of structural and geometrical parameters selected, the scleral stiffness was identified to have the greatest influence on the ONH and the strains in the LC, and the principal strain in the LC varied inversely with the scleral stiffness [28]. These results indicated that the mechanical property of the sclera is a crucial factor that can affect the LC. This may be a reason that glaucoma patients were found to have higher ocular rigidity [32].

It has also been found that the ocular tissue mechanical elastic properties, including that of the sclera and the LC, vary with age. Both the sclera and the LC were found to be stiffer and have higher elastic moduli in more aged eyes [33, 34]. Since there is a higher prevalence
of glaucoma in the aged population, the age-stiffening properties of sclera can be an important cause of glaucoma. People with stiffened ocular tissues may potentially be more susceptible to optic nerve damage from IOP elevation.

1.3 Research gaps

Although glaucoma is associated with IOP elevation, it is found that the extent of glaucomatous visual field loss does not have good statistical correlation with the existing IOP measurements, as shown in Figure 1-11 [35]. This implies that the risk assessment by current tonometry cannot clearly distinguish the eyes that are more susceptible to optic nerve damage. This may be due to the inaccuracy in current IOP measurement and the current measurement systems being unable to offer individual specific assessment criteria for eyes with different biomechanical properties.

1.3.1 Inaccuracy in existing IOP measurement

Precise measurement of the IOP is crucial for the risk assessment and management of glaucoma. Simply an IOP reduction of 1 mmHg can lead to a 10% reduction in the risk of visual field deterioration [36]. A measurement error of several mmHg in the IOP is critical in the risk assessment and the treatment of glaucoma, but the current gold reference standard of IOP measurement, Goldmann Applanation Tonometry (GAT), does not have the ability to measure the IOP accurately. The GAT IOP readings can be greatly affected by the variations in the central corneal thickness, the corneal radius of curvature and the corneal elastic modulus [37-44]. The measurement error caused solely by the corneal biomechanical property can be up to 15 mmHg (Figure 1-12) [37].

The basic principle of GAT relies on the Imbert-Fick Law [25], which states that the internal fluid pressure that acts on an infinitesimally thin, elastic and homogenous membrane
sphere is equal to the pressure that is used to flatten a small portion of the membrane:

\[ W = A \cdot IOP \]  \hspace{1cm} (1.1)

where \( W \) is the force to applanate the corneal surface and \( A \) is the applanated area. The applicability of the Imbert-Fick law is however limited due to several reasons:

1. The cornea is not infinitely thin and clinical evidence has demonstrated that the accuracy of the IOP measurement can be significantly affected by the variation of the central corneal thickness [37, 45-49].

2. The individual variations in corneal mechanical properties in reality can lead to huge measurement errors [37, 50, 51].

3. Since the actual cornea is wet in nature, when the flat tonometer head comes into contact with the cornea, the surface tension \( s \) from the tear film on the cornea exerts an inward force onto the head and pulls it toward the cornea. At the same time, an outward bending resistance force \( b \) is generated by the cornea. Goldmann and Schmidt [25] expected an empirical balance between the surface tension and the resistance force of the corneal membrane when the applanation contact area is equal to 7.35mm\(^2\). The surface tension of the tear film is approximately constant among subjects, but the force that is used to deform the cornea depends on the central corneal thickness, the corneal radius of curvature and the corneal mechanical response. Thus, the applanation contact area where the tear film surface tension force equals the resistance force generated by the cornea is not universal for different eyes due to the individual variations in corneal properties. The modified Imbert-Fick Law is:

\[ W + s = A \cdot IOP + b \] \hspace{1cm} (1.2)

where \( s \) is the surface tension of the tear film and \( b \) is the outward resistance force generated by the corneal membrane.

Therefore, the GAT IOP reading is not accurate when the corneal properties of an eye
deviate from that of the general population. For instance, the IOP of elderly people with age-stiffened corneas [52] or people with inherently stiffened corneas can be over-estimated by GAT [37], while the IOP of the patients whose cornea is surgically thinned by laser-assisted in situ keratomileusis (LASIK), a refractive surgery for correcting myopia, tends to be under-estimated [53, 54]. There is currently no method for correcting the measured IOP accordingly. An IOP measurement method that can account for the variations in the corneal biomechanical properties is needed.

1.3.2 Inability to incorporate an individual specific risk assessment criterion

A problem in current glaucoma risk assessment is that clinicians typically compare the measured IOP with a general threshold IOP (21 mmHg) for every individual. There is no individual specific risk assessment criterion for different eyes. Nevertheless, it has been found that different eyes have different susceptibility to glaucomatous damage. NTG or ocular hypertension without glaucomatous damage cannot be properly explained by IOP elevation at the moment. These factors mean that a single threshold IOP level cannot identify all high-risk patients.

The susceptibility of an eye to glaucomatous damage is indeed affected by the biomechanical properties of the ocular tissues. Both the glaucoma risk and the mechanical stiffness of ocular tissues increase with age. The variation in ocular biomechanical properties can alter the biomechanics in the ONH region and affect how the tissues in the ONH behave when they are subject to loadings. Understanding the ocular biomechanics would be one of the important keys in developing individual specific risk assessment criteria for glaucoma. As yet, an understanding of the biomechanics concerning the pathology of the glaucomatous nerve damage is still elusive. There is currently no theoretical study quantitatively examining the biomechanical reason for the clinical observations that peripheral vision loss precedes
central vision loss in glaucoma.

Moreover, most of the tests that currently measure the mechanical properties of ocular tissues, such as tensile strip tests [55, 56] and inflation tests [57-59], remain invasive or destructive and are not practical to be used on living patients in clinics. Consequently, there is currently no clinical study investigating how the ocular biomechanical properties affect the susceptibility to glaucomatous optic nerve damage. The general threshold IOP for the risk assessment of glaucoma in clinics today, 21 mmHg, remains in doubt for people with age-stiffened and inherently stiffened eyes. Individual specific glaucoma risk assessment criteria that can account for the variations in ocular biomechanical properties is needed.

1.4 Problem statement

Tonometry is the primary risk assessment method for glaucoma. The measured IOP in tonometry is generally compared to a threshold IOP level (21 mmHg) as a reference to determine if the subject is at risk. Both the measured IOP and the threshold IOP, however, ignore the individual variations of the ocular tissue biomechanical properties in human eyes, causing the existing glaucoma risk assessment to fail to accurately distinguish the eyes that are at risk. The inclusion of individual biomechanical properties is needed to improve the accuracy of the measured IOP and the clinical relevance of the threshold IOP, but a non-invasive method for characterizing ocular tissues in clinics is currently unavailable.

1.5 Scope of the thesis

To improve the clinical relevance of tonometry, the development of an IOP measurement technique that can account for the individual biomechanical properties and a proposal for individual-specific risk assessment criteria incorporating the consideration of ocular tissue biomechanics are presented in this thesis:
As the IOP measurements from the gold standard GAT cannot account for the variations in corneal properties, the accuracy of the IOP reading is inadequate. To improve IOP measurement accuracy, an instrumented corneal properties-independent intraocular pressure measurement method is developed in Chapter 2. The method measures the corneal stiffness and the IOP simultaneously, such that the variations in corneal geometries and mechanical properties can be taken into account in the IOP measurement. The experimental method and IOP analysis were tested on ex vivo porcine eyes. The measured IOPs were compared with the manometer-controlled IOPs to determine the validity of the method. The analysis showed that the IOP measurement errors could be more than 50% without corneal stiffness correction, but were significantly reduced to less than 10% by incorporating the corneal stiffness into the IOP measurement. This technique may help improve the accuracy of IOP measurement for better glaucoma risk assessment and management, in particular for elderly people with age-stiffened eyes and LASIK patients with surgically thinned corneas.

A general threshold IOP (21 mmHg) is commonly used in clinics for the glaucoma risk assessment of every subject, but it has been found that different eyes have different susceptibility to optic nerve damage caused by IOP elevation. The pathology of glaucoma is indeed closely related to the biomechanics in the ONH region of the eyes, where both the loading (i.e. IOP) and the elasticity of the ocular tissues are important components. The variations in ocular biomechanical properties among people would affect the level of susceptibility of an individual eye to glaucomatous damage. Chapter 3 therefore presents a finite element analysis (FEA) to quantitatively examine the effect of the biomechanical properties of ocular tissues on the LC, the primary site of glaucoma in the ONH region. The aim is to determine if elderly people with stiffened ocular tissues will suffer more optic nerve damage compared to un-stiffened eyes under the same IOP, and to estimate the significance of the level of damage due to tissue stiffening. From our model, the Tresca’s shear failure
criterion can phenomenologically explain that stiffer eyes are more subject to damage by IOP elevation. The results showed that ocular tissue stiffening is a significant factor in accounting for higher glaucoma prevalence in the elderly. *In vivo* measurement of ocular tissue stiffness and individual adjustment on the threshold IOP are therefore recommended to improve diagnostic accuracy and to identify high-risk glaucoma patients for alternate treatment to arrest vision loss.

Even though ocular tissue biomechanical properties may be important in glaucoma, a technique for measuring these non-invasively in clinics is currently unavailable. A non-invasive indentation technique for measuring the biomechanical properties of ocular tissues is now developed, such that the *in vivo* mechanical stiffness of human corneas and sclerae can be acquired non-invasively for studying the relation between glaucoma and ocular tissue biomechanical properties. The method was first tested on porcine eyes, as presented in Chapter 4. Human cadaver eyes were then tested, as shown in Chapter 5, and the results showed that the indentation technique can be adapted to characterize human ocular tissue biomechanical properties. A preliminary clinical study on human eyes was conducted, and this is presented in Chapter 6. The novel ocular mechanical property measurement method may potentially be used for future individual adjustment of threshold IOP for better risk assessment of glaucoma. This could help identify people who are more susceptible to glaucoma and help arrest their vision loss by earlier treatment.
Figures

Figure 1-1. Schematic diagram of the eye [60].

Figure 1-2. Schematic diagram of the optic nerve head [61].
Figure 1-3. Schematic diagram of a myopic eye [62].

Figure 1-4. Vision illustration of eyes with glaucoma [63].

Figure 1-5. IOP distribution in non-glaucoma (N) and glaucoma (G) population [9].
Figure 1-6. The prevalence of OAG for different ethnic groups [13].

Figure 1-7. The prevalence of ACG for the different ethnic groups [13].
Figure 1-9. Micrography of the ONH region from a normal human eye (a) fixed at 5 mmHg (b) fixed at 50 mmHg (Gomori’s trichrome, ×35) [3].
Figure 1-10. Micrography of the LC from a normal human eye (a) fixed at 5 mmHg (b) fixed at 50 mmHg (Gomori’s trichrome, ×140) [3].
Figure 1-11. IOP vs visual field mean deviation (MD) index in primary open-angle glaucoma (POAG) group [35].

Figure 1-12. Theoretical prediction of the influence of the corneal mechanical property on IOP measurement [37].
Table 1-1. Intraocular pressure and the prevalence of open-angle glaucoma (OAG) [4]

<table>
<thead>
<tr>
<th>IOP (mmHg)</th>
<th>Prevalence (%)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>16 – 18</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>19 – 21</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>22 – 24</td>
<td>8.3</td>
<td>12.8</td>
</tr>
<tr>
<td>25 – 29</td>
<td>8.3</td>
<td>12.8</td>
</tr>
<tr>
<td>30 – 34</td>
<td>25.4</td>
<td>39.0</td>
</tr>
<tr>
<td>≥35</td>
<td>26.1</td>
<td>40.1</td>
</tr>
</tbody>
</table>
Chapter 2

2 Development of individual-specific tonometry

Abstract

Intraocular pressure (IOP) monitoring is important in the diagnosis and management of glaucoma. The measurement of IOP is affected by corneal properties, but the effect of corneal stiffness on IOP measurement is unaccounted for in pressure measurement instruments such as the Goldmann Applanation Tonometer (GAT). A new instrumented non-invasive indentation tonometry that can measure \( IOP_{IST} \), a corneal stiffness-corrected intraocular pressure is developed. The inter-individual corneal variations of 12 porcine eyes \( ex \ vivo \) were independently characterized; and their true intraocular pressure, \( IOP_T \)'s, were set using a manometer before indentation using the new indentation tonometry. Analyses of the load-displacement data showed that porcine corneal stiffness varied more than five times from 0.045 to 0.253 N/mm. Analysis showed that, without individual stiffness correction, the inter-individual variation of \( IOP_{GAT} \) can vary up to 8 mmHg from \( IOP_T \) at 15mmHg; the error becomes larger at high \( IOP_T \). In comparison, when corneal stiffness is accounted for, \( IOP_{IST} \) has a significantly smaller error of 1.82±1.70 mmHg for \( IOP_T \) between 12 – 40 mmHg than \( IOP_{GAT} \). The results showed that the new tonometry method successfully accounted for inter-individual variations in IOP measurement.

2.1 Introduction

Intraocular pressure (IOP) monitoring is important in the diagnosis and management of glaucoma. A new trans-scleral method [64] has been developed to increase measurement convenience, but methods to improve the accuracy of IOP measurement have not been
successfully developed [65]. The current gold standard of tonometry, the Goldmann Applanation Tonometry (GAT), is known to be affected by individual variations of the central corneal thickness, the corneal radius of curvature and the corneal elastic modulus [37-44, 65]. While healthy eyes normally have an IOP lower than 21 mmHg, up to 15 mmHg in measurement error had been attributed to individual variations in the corneal biomechanical properties [37]. Amongst the property variations, studies showed that the corneal tangent modulus of an individual varies with the individual’s IOP (Figure 2-1) [66]. This suggests that IOP measurements not only vary with individuals, but also vary with the individual’s IOP at the time of the measurement. Since the corneal stiffness increases with IOP, the measurement error for glaucoma sufferers with high IOP would be higher than the measurement error at low IOP, even for the same individual.

The measurement error from ignoring the individual corneal property contribution can be understood by examining the theoretical basis of conventional tonometric methods. The GAT method is derived from the modified Imbert-Fick Law, which is a force balance between the measured applied force \( F \), surface tension force of the tear film \( s \), pressure force \( A \cdot IOP \) and corneal resistance force \( b \) [25],

\[
F + s = A \cdot IOP + b, \tag{2.1}
\]

where \( A \) is the applanation contact area between the GAT probe and the cornea. GAT empirically requires the operator to applanate the cornea to an area \( A_{GAT} = 7.35 \text{mm}^2 \) such that the surface tension force \( s \) is counter-balanced by the corneal resistance \( b \). In this condition, the IOP can be computed via,

\[
IOP_{GAT} = \frac{F_{GAT}}{A_{GAT}}, \tag{2.2}
\]
where $F_{GAT}$ is the indentation force measured by GAT. The method is accurate for a patient with a cornea resistance $b$ equal to $s$. Error arises when $b$ and $s$ are not equal. From Young [67], the corneal resistance force $b$ is related to the corneal radius of curvature $r$, the corneal thickness $t$, and the corneal tangent modulus $E$ (see Eq.(2.7)). The relation shows that the doubling of $E$ would double $b$. Individual and IOP induced variations of $E$ would result in the deviation of $b$ away from $b$ assumed in GAT, and lead to a large measurement error in GAT [37]. To account for variations in inter-individual corneal properties, a new individual-specific tonometry (IST) is developed (US Provisional Patent Application (US 61/675,361)). The validity of the method was tested on porcine eyes ex vivo. The $IOP_{IST}$ measured using IST was compared with the true $IOP_T$ from the manometer to determine whether the new method can account for the corneal properties’ influence.

2.2 Methods

2.2.1 Methodology of individual-specific tonometry

In GAT, the eye is applanated to a constant $A_{GAT} = 7.35\text{mm}^2$. The surface tension force $s$ which depends on the size of the contact area is fixed when the applanation area is fixed. Since GAT assumes that $b = s$, the corneal resistance $b$ is also fixed. In actual eyes, the $E$ of the cornea changes from individual to individual and is dependent on the pressure in the eye at the time of the measurement. During applanation, $b$ may become equal to $s$ at an arbitrary $A^*$, which often is not $A_{GAT}$. The area $A^*$ at which the individual’s corneal resistance force counter-balances the surface tension force, can be computed from the corneal

\footnote{A table of variable definitions (Table 2-2) is provided to enhance readability.}

\[ \text{Table of variable definitions (Table 2-2) is provided to enhance readability.} \]
load-displacement curve.

In individual-specific tonometry (IST), the corneal resistance that exactly counter-balances the surface tension force at a specific applanation area $A^*$ is $b^*$, such that,

$$b^* = s^*.$$  \hspace{1cm} (2.3)

where $s^*$ is the specific surface tension force at $A^*$. Accordingly, Eq.(2.1) becomes,

$$IOP_{IST} = \frac{F^*}{A^*},$$  \hspace{1cm} (2.4)

where $F^*$ is the applied force at $A^*$ and $IOP_{IST}$ is the individual-specific intraocular pressure. The starred quantities ($F^* ; A^* ; b^*$) are uniquely related to each other. They cannot be determined from single load tonometry tests such as GAT. Instead, they are determined from the indentation load-displacement curve (Figure 2-3).

In instrumented corneal indentation, the indenter contact with the cornea is initially a point. With indentation, the contact becomes a partial contact and then full contact where the entire flat tip is in contact with the cornea even with increasing $\delta$. The change in contact force as a function of indent depth $\delta$ can be obtained by differentiating Eq.(2.1),

$$\frac{dF}{d\delta} + \frac{ds}{d\delta} = \frac{d}{d\delta}(A \cdot IOP) + \frac{db}{d\delta}.$$  \hspace{1cm} (2.5)

In the full contact regime, where the contact area no longer changes as a function of $\delta$, the surface tension force $s$ and $A \cdot IOP$ are independent of $\delta$. Eq.(2.5) can be simplified to,
\[
\frac{dF}{d\delta_{lc}} = \frac{db}{d\delta_{lc}},
\]

where the term on the left is the slope of the load-displacement curve in the full contact regime (see Appendix for details).

From Young [67], the corneal resistance force \( b \) is,

\[
b = \frac{E \cdot t^2}{a(r - t / 2)\sqrt{1 - \nu^2}} \delta.
\]

where \( E \) is the corneal tangent modulus, \( r \) is the corneal radius of curvature, \( t \) is the corneal thickness, \( \nu (\approx 0.5 \) [68]) is the Poisson’s ratio of the cornea and \( a \) is the corneal geometric coefficient. The corneal geometric constant \( a \) is determined from \( \mu \),

\[
\mu = \frac{D}{2} \left[ \frac{12(1 - \nu^2)}{(r - t / 2)^2 t^2} \right]^{1/4},
\]

where \( D \) is the diameter of the contact area between the indenter and the cornea. The relation between \( a \) and \( \mu \) is given in Table 2-1 [67].

<table>
<thead>
<tr>
<th>( \mu )</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
<th>1.2</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a )</td>
<td>0.433</td>
<td>0.431</td>
<td>0.425</td>
<td>0.408</td>
<td>0.386</td>
<td>0.362</td>
<td>0.337</td>
<td>0.311</td>
<td>0.286</td>
</tr>
</tbody>
</table>
Differentiating Eq. (2.7) with respect to the corneal indentation depth \( \delta \) at full contact gives,

\[
\frac{db}{d\delta} = \frac{E \cdot t^2}{a(r-t/2)\sqrt{1-v^2}}.
\] (2.9)

The change in corneal resistance force at full contact can be related to the change of the corneal resistance force at an arbitrary \( \delta \) via,

\[
\frac{db}{d\delta} \bigg|_{a_c} = \frac{db}{d\delta} \bigg|_{a_s}.
\] (2.10)

Mathematically, the corneal resistance force \( b^* \) can be determined from the slope via,

\[
b^* = \frac{db}{d\delta} \bigg|_{b^*}.
\] (2.11)

where \( \delta^* \) is the corneal indentation depth when the corneal resistance \( b^* \) exactly counter-balances the surface tension force \( s^* \). Combining Eq. (2.6), and (2.10) and (2.11) for indentation at \( \delta^* \), \( b^* \) is,

\[
b^* = \frac{dF}{d\delta} \bigg|_{b^*} \frac{a_c}{a_s} \delta^*.
\] (2.12)

Since \( s \) is linearly proportional to the circumference of the cornea-indenter contact, the specific surface tension force \( s^* \) can be determined from,

\[
s^* = s_{GAT} \frac{D^*}{D_{GAT}}.
\] (2.13)
where \( D_{GAT} \) is 3.06mm and \( s_{GAT} \) is the surface tension force of the tear film at \( A_{GAT} \). Before the indenter is in full contact with the cornea, i.e., \( D^* < D_{indenter} \),

\[
D^* = \sqrt{8r\delta^*}
\]  
(2.14)

and

\[
A^* = 2\pi r\delta^*.
\]  
(2.15)

Substituting Eq.(2.14) into Eq.(2.13) gives,

\[
s^* = s_{GAT} \cdot \frac{\sqrt{8r\delta^*}}{D_{GAT}}.
\]  
(2.16)

Further substituting Eq.(2.12) and Eq.(2.16) into Eq.(2.3) gives,

\[
\left. \frac{dF}{d\delta} \right|_{\delta^*} \frac{a_{fc}}{a_{\delta^*}} - s_{GAT} \frac{\sqrt{8r\delta^*}}{D_{GAT}} = 0
\]  
(2.17)

For GAT applanation, the equation becomes,

\[
\left. \frac{dF}{d\delta} \right|_{GAT} \frac{a_{GAT}}{a_{\delta^*}} - s_{GAT} \frac{\sqrt{8r\delta^*}}{D_{GAT}} = 0
\]  
(2.18)

Using \( dF/d\delta \) from the load-displacement data and the corneal geometry data (i.e. \( r \) and \( t \)), \( \delta^* \), \( A^* \) and \( b^* \) can be determined by solving Eq.(2.17), Eq.(2.15) and Eq.(2.12) for eyes indented at different IOPs.

Mechanically, the cornea deforms nonlinearly [39, 69], and the corneal tangent modulus
has been shown to vary linearly with IOP [66]. The constitutive relation between \( b^* \) and the pressure force \( A^* \cdot IOP \) can be formulated as,

\[
\gamma = \frac{A^* \cdot IOP}{b^*},
\]

(2.19)

where \( \gamma \) is a material constant (see Appendix for details). \( \gamma \) can be determined by fitting Eq.(2.20) onto a plot of \( A^* \cdot IOP \) versus \( b^* \) (i.e. Figure 2-4 in the Results section). Substituting \( F^* = A^* \cdot IOP \) and Eq.(2.19) into Eq.(2.4) gives,

\[
IOP_{IST} = \frac{\gamma b^*}{A}.
\]

(2.20)

Substituting Eq.(2.15) into Eq.(2.20), and rewriting in terms of the slope of the load-displacement data during indentation, gives,

\[
IOP_{IST} = \frac{\gamma}{2\pi r} \left. \frac{dF}{d\delta} \right|_{\delta_c} \frac{a_c}{a_s},
\]

(2.21)

where \( IOP_{IST} \) is a function of the individual’s corneal stiffness \( dF/d\delta \) at full contact.

For reference, the principal differences between GAT and the IST method developed in this study are shown in Table 2-3. In comparison, standard GAT that determines the IOP from a single load does not account for the individual’s corneal stiffness. By using the slope of the load-displacement curve in the full contact regime, the corneal stiffness is now accounted for in \( IOP_{IST} \).
### Table 2-2. Table of variable definitions.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IOP$</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>$IOP_{GAT}$</td>
<td>Intraocular pressure measured by GAT</td>
</tr>
<tr>
<td>$IOP_{IST}$</td>
<td>Intraocular pressure measured by IST</td>
</tr>
<tr>
<td>$IOP_T$</td>
<td>True intraocular pressure</td>
</tr>
<tr>
<td>$F$</td>
<td>Applied force by indenter</td>
</tr>
<tr>
<td>$F_{GAT}$</td>
<td>Applied force by GAT when $A=A_{GAT}$</td>
</tr>
<tr>
<td>$F^*$</td>
<td>Applied force by indenter when $A=A^*$</td>
</tr>
<tr>
<td>$s$</td>
<td>Surface tension of the tear film</td>
</tr>
<tr>
<td>$s_{GAT}$</td>
<td>Surface tension of the tear film when $A=A_{GAT}$</td>
</tr>
<tr>
<td>$s^*$</td>
<td>Surface tension of the tear film when $A=A^*$</td>
</tr>
<tr>
<td>$A$</td>
<td>Applanation area</td>
</tr>
<tr>
<td>$A_{GAT}$</td>
<td>Applanation area assumed by GAT</td>
</tr>
<tr>
<td>$A^*$</td>
<td>Applanation area at which individual’s corneal resistance force counter-balances the surface tension force ($b=s$)</td>
</tr>
<tr>
<td>$b$</td>
<td>Corneal resistance force</td>
</tr>
<tr>
<td>$b^*$</td>
<td>Corneal resistance force when $A=A^*$</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Indentation depth</td>
</tr>
<tr>
<td>$\delta_{GAT}$</td>
<td>Indentation depth when $A=A_{GAT}$</td>
</tr>
<tr>
<td>$\delta^*$</td>
<td>Indentation depth when $A=A^*$</td>
</tr>
<tr>
<td>$D$</td>
<td>Diameter of the applanation</td>
</tr>
<tr>
<td>$D_{\text{indenter}}$</td>
<td>Diameter of the indenter (i.e. $D$ at full contact)</td>
</tr>
<tr>
<td>$D^*$</td>
<td>Diameter of the applanation when $A=A^*$</td>
</tr>
<tr>
<td>$E$</td>
<td>Corneal tangent modulus</td>
</tr>
<tr>
<td>$r$</td>
<td>Corneal radius of curvature</td>
</tr>
<tr>
<td>$t$</td>
<td>Corneal thickness</td>
</tr>
<tr>
<td>$a$</td>
<td>Corneal geometric coefficient in Eq.(2.7)</td>
</tr>
<tr>
<td>$a_{fc}$</td>
<td>Corneal geometric coefficient in Eq.(2.7) at full contact</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$\alpha_s$</td>
<td>Corneal geometric coefficient in Eq.(2.7) when $A=A'$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Material constant accounting for corneal properties dependence on IOP</td>
</tr>
<tr>
<td>$\frac{db}{d\delta}$</td>
<td>Corneal stiffness</td>
</tr>
<tr>
<td>$\frac{dF}{d\delta}$</td>
<td>Slope of the load-displacement curve</td>
</tr>
<tr>
<td>$\frac{dF}{d\delta_{bc}}$</td>
<td>Slope of the load-displacement curve at full contact</td>
</tr>
</tbody>
</table>

Table 2-3. Comparison between GAT and IST.

<table>
<thead>
<tr>
<th><strong>Goldmann Applanation Tonometry (GAT)</strong></th>
<th><strong>Individual-specific tonometry (IST)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Single-point static load measurement</td>
<td>- Continuous load-displacement dynamic measurement by instrumented tonometric indentation</td>
</tr>
<tr>
<td>- GAT approximates that $b=s$ when $A=7.35\text{mm}^2$ for every eye, such that $IOP_{GAT} = \frac{F_{GAT}}{7.35}$.</td>
<td>- A specific area $A'$ where $b=s$ is found for each eye, such that $IOP_{IST} = \frac{F'}{A'}$, where $F'$ is the applied force at $A'$.</td>
</tr>
<tr>
<td>- Assumptions:</td>
<td>- $A'$ is calculated from:</td>
</tr>
<tr>
<td>- Corneal radius of curvature (7.8 mm) [4]</td>
<td>- Individual corneal radius of curvature</td>
</tr>
<tr>
<td>- Corneal thickness (0.52 mm) [4]</td>
<td>- Individual corneal thickness</td>
</tr>
<tr>
<td>- Corneal stiffness (0.0271 N/mm) – obtained in this study</td>
<td>- Individual corneal stiffness</td>
</tr>
</tbody>
</table>
2.2.2 Experimental procedure

12 fresh *ex vivo* porcine eyes were tested in this study. The porcine eyes were obtained from a local abattoir. The experiments were conducted within 12 hours of the animals being sacrificed. The eyes were kept moist at a low temperature (4 °C) inside an insulated bucket. The central corneal thickness and corneal radius of curvature of the porcine eyes were measured using photographs taken using a Leica DFC295 digital camera attached to a Leica M205C stereomicroscope (Leica Microsystems, Wetzlar, Germany). The mean corneal radius of curvature and the mean central corneal thickness of the eyes were 7.40±0.67 mm and 1.10±0.15 mm respectively².

Porcine eyes with the muscle and the adipose tissue attached were used. The anterior chamber was cannulated³ (2 mm from the limbus). The eye was filled with saline using a hypodermic needle (Figure 2-2) connected to a saline-filled manometer to control the IOP. A pressure transducer (OPP-M400 packaged pressure sensor, Opsens Inc., Canada) was inserted into the system to measure the IOP independently. The IOP varied from 12 to 40 mmHg.

The eye was mounted on the test jig and aligned in place underneath the indenter for indentation, and the eye was kept moist using saline solution with an eye dropper throughout

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² mean ± standard deviation of the mean calculated from all tested porcine eyes; standard deviation on a single measurement basis is less than 0.05mm

³ Indentation experiments were performed on eyes by cannulating the vitreous chamber through the sclera. Negligible differences were found in the load-displacement data between cannulating the anterior chamber through the cornea and cannulating the vitreous chamber through the sclera. On this basis, cannulations in our experiments were done through the anterior chamber.
the procedure. The indentation was conducted on a universal testing machine (MTS Alliance RT/5, USA). A 5-mm-diameter \( D_{in} \) cylindrical indenter was screw-mounted onto a 10N load cell (MTS 100-090-795). The load cell, in turn, was screw-mounted onto the crosshead of the machine. The machine was set to indent at 20 mm/min to depth to 1.0 mm for all eyes, and the load-displacement curves after indentations were collected for analysis.

### 2.2.3 Effect of corneal properties on IOP

GAT does not account for corneal properties. If an individual’s corneal stiffness is different from the standard GAT stiffness, the IOP measured using GAT will depart from the actual pressure in the eye chamber. The magnitude of the measurement error arising from excluding the corneal property is estimated below.

The GAT applanation area \( A_{GAT} \) 7.35 mm\(^2\) is valid for eyes with \( b = s \) and with radius and thickness at population average \( r_{GAT} \) and \( t_{GAT} \) of 7.80mm and 0.52mm, respectively \([38]\). Since corneal stiffness is ignored in GAT, an assumption of a standard geometry for all eyes would lead to constant stiffness for all eyes in GAT measurement. The corneal stiffness \( \frac{db}{d\delta_{GAT}} \) can be estimated from Eq.(2.11) and Eq.(2.15) via,

\[
\left| \frac{db}{d\delta_{GAT}} \right| \frac{A_{GAT}}{2\pi r_{GAT}} - s_{GAT} = 0. \tag{2.22}
\]

From this equation, the standard GAT stiffness assumed for all eyes in GAT measurement \( \frac{db}{d\delta_{GAT}} \) is 0.027 N/mm.

The influence of variable corneal stiffness on \( IOP_{GAT} \) can be then determined by combining Eq.(2.1) and Eq.(2.2) such that,
\[ IOP_GAT = \frac{IOP \cdot A_GAT + \frac{db}{d\delta} \delta_GAT - s_GAT}{A_GAT}. \] (2.23)

Eq.(2.23) is suitable for human eyes, but can be modified to estimate the \( IOP_GAT \) for porcine eyes to lend insight into the effect that the constant corneal stiffness assumption has on IOP measurement. The manometer pressure \( IOP_T \) and the corneal stiffness \( \frac{db}{d\delta} \) from the indentation measurement on porcine eyes were used in Eq.(2.23) to compute the \( IOP_GAT \) via,

\[ IOP_GAT = \frac{IOP_T \cdot A_GAT + \frac{db}{d\delta} \delta_GAT - s_GAT}{A_GAT}, \] (2.24)

for the porcine eyes indented in this study.

2.3 Results

2.3.1 Experimental data on porcine eyes

A typical load-displacement curve on a pressurized porcine eye is shown in Figure 2-3. The slope of the loading portion of the load-displacement data in the full contact regime (displacement: 0.45 to 1 mm, linear regression: \( R^2=0.999 \)) is linear. Analysis of the data showed that the full contact indentation slope, i.e., \( \frac{dF}{d\delta} \) varied from 0.045 to 0.253 N/mm for the porcine eyes tested. The material constant \( \gamma \) for \textit{ex vivo} porcine eyes was determined to be 1.73 (Figure 2-4). The \( IOP_{IST} \) of the specimen eyes, which accounted for the corneal properties of the eyes, was determined using Eq.(2.21).

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\(^4\) Indentation speed corrected
The accuracy of the experimentally determined $IOP_{IST}$ and the estimated $IOP_{GAT}$ are shown in Figure 2-5. The Bland-Altman plot showing the difference between $IOP_{IST}$ and $IOP_T$ as a function of $IOP_T$ is shown in Figure 2-6. The differences between $IOP_{IST}$ and $IOP_T$, and between $IOP_{GAT}$ and $IOP_T$, are also plotted as a function of the corneal stiffness (Figure 2-7). The average errors for the tested eyes over the tested range were $1.82 \pm 1.70$ mmHg and $3.71 \pm 2.17$ mmHg for IST and GAT, respectively. $IOP_{GAT}$ is shown to increasingly overestimate $IOP_T$ with high corneal stiffness. On the other hand, $IOP_{IST}$ is shown to be independent of $IOP_T$. These results showed that the intraocular pressure can be more accurately characterized by IST than by GAT.

### 2.3.2 Effect of corneal properties on GAT in human eyes

$A^*$, the individual corneal equilibrium applanation area determined using Eq.(2.15), is plotted in Figure 2-8 as a function of the corneal stiffness. The plot shows that the individual equilibrium applanation area becomes significantly different from the constant $A_{GAT}$ when corneal stiffness departs from the GAT average stiffness.

Using Eq.(2.23), the effect of corneal stiffness on $IOP_{GAT}$ for human eyes with $r=7.8$mm and $t=0.52$mm at fixed 15mmHg is shown in Figure 2-9. The plot shows that the $IOP_{GAT}$ varied from $IOP_T$ by up to 8 mmHg because of variations in individual corneal stiffness.

---

5 The Bland-Altman plot showing the difference between $IOP_{GAT}$ and $IOP_T$ against $IOP_T$ is not given because $IOP_{GAT}$ was estimated by using the accurate $IOP_T$ with Eq.(2.24). The corresponding standard deviation in the Bland-Altman plot would not fully reflect the variation of GAT measurement in reality.
2.4 Discussion

Similar overestimates in $IOP_{GAT}$ from ignoring individual corneal stiffness differences would be expected in humans where the cornea is known to stiffen with age. Corneal stiffness is dependent on both age and IOP [39, 70]. The corneal stiffness of a 90-year-old eye is approximately double that of a 60-year-old eye [39]. Individual variations in the corneal stiffness alone can result in IOP measurement errors up to 8 mmHg at an IOP of 15 mmHg (Figure 2-9).

Independent of age, intraocular pressure also has a significant effect on the corneal stiffness. The corneal stiffness of an eye with IOP at 30 mmHg is approximately double that of an eye with IOP at 15 mmHg [70]. When individual differences in corneal stiffness are unaccounted for in IOP measurement such as in Goldmann Applanation Tonometry, large non-negligible error from age and IOP stiffening effects would result.

From the results, it should be noted that the standard deviation in $IOP_{GAT}$ at a fixed $IOP_T$ is smaller than that in $IOP_{IST}$ (Figure 2-5). $IOP_{GAT}$ is a function of $F_{GAT}$, which in turns depends on the corneal stiffness, the pressure inside the eye ($IOP_T$), and the accuracy and stability of the required applanation. In actual tests on humans, $F_{GAT}$ is directly acquired from the tonometer. In our study, there was no GAT for porcine eyes, and $IOP_{GAT}$ had to be estimated mathematically using (Eq.(2.24)) based on the load-displacement data. As a consequence, the errors were semi-experimental since various experimental variations such as applanation inaccuracies were not reflected in the estimate. The error bars shown in the calculated porcine $IOP_{GAT}$ should not be construed as true standard deviation from an experimental porcine GAT test. Error analysis should be applied later when data from $IOP_{GAT}$ are available from clinical tests at a later stage.

$IOP_{IST}$ was obtained by indenting the cornea by 1.0mm in this study. A comparison of $IOP_{IST}$ with $IOP_T$ showed good agreement. This showed that IOP variations during indentation
had minimal effect on $IOP_T$ measurement.

The present study showed that the $IOP_{IST}$ measured using IST can effectively account for variation in corneal stiffness and the results were benchmarked with the manometer pressure (Figure 2-5). By accounting for individual corneal variation, the error in IST is significantly lower than the error in GAT (Figure 2-7). This exclusion error can be avoided by using the individual specific tonometry (IST) developed in this study, and the new IST can potentially improve the IOP measurement accuracy for aged and pressure stiffened eyes. Further studies will be performed to improve the precision of IST and test its validity on human eyes.

2.5 Conclusions

A new indentation method to measure the IOP was developed and validated on ex vivo porcine eyes. While the corneal stiffness ranged from 0.045 to 0.253 N/mm, the accuracy of the IOP measured using individual-specific tonometry (IST) successfully accounted for the stiffness variation and successfully measured the IOP more accurately than GAT. The measurement error of the new method is lower than the error in GAT, where corneal stiffness variation is unaccounted for in the calculation. The results suggest that individual-specific tonometry (IST) can successfully account for individual variations in corneal stiffness in the assessment of the pressure state of the eye.
Appendix: Details of derivation from Eq.(2.5) to Eq.(2.6)

The area of corneal contact does not change when the indenter tip area is in full contact with the cornea. Once full contact is reached, the applanation area $A$ becomes constant and is independent of $\delta$. As a result, $\left. \frac{dA}{d\delta} \right|_{fc} = 0$. Since the applanation area is constant with a constant perimeter, the surface tension force no longer changes with respect to further indentation and $\left. \frac{ds}{d\delta} \right|_{fc} = 0$. Thus, Eq.(2.5) can be simplified to $\left. \frac{dF}{d\delta} \right|_{fc} = A_{fc} \cdot \left. \frac{d}{d\delta} (IOP) \right|_{fc} + \left. \frac{db}{d\delta} \right|_{fc}$.

From the experiment, $\left. \frac{d}{d\delta} (IOP) \right|_{fc}$ after full contact was between 1 to 3 mmHg/mm during indentation. Comparison of $\left. \frac{dF}{d\delta} \right|_{fc}$ and $A_{fc} \cdot \left. \frac{d}{d\delta} (IOP) \right|_{fc}$ showed that $A_{fc} \cdot \left. \frac{d}{d\delta} (IOP) \right|_{fc}$ was only about 1-3% of $\left. \frac{dF}{d\delta} \right|_{fc}$. Since $A_{fc} \cdot \left. \frac{d}{d\delta} (IOP) \right|_{fc}$ is negligible at full contact, Eq.(2.5) becomes Eq.(2.6). Experimentally, $IOP_{IST}$ determined while assuming the term is negligible is shown to be in good agreement with $IOP_T$ (Figure 2-5). This confirmed that Eq.(2.6) is sufficient for the computation of $IOP_{IST}$. 
Appendix: Material constant accounting for corneal properties dependence on IOP

The stress-strain behavior of the cornea is nonlinear (Figure 2-10). This behavior can be modeled using an exponential form [5, 15],

$$\sigma = \alpha \left( e^{\kappa \epsilon} - 1 \right), \quad (2.25)$$

where $\sigma$ is stress, $\epsilon$ is strain, $\alpha$ and $\kappa$ are two material constants. This is the constitutive law of the cornea. Differentiating Eq.(2.25) with respect to strain $\epsilon$ gives the elastic tangent modulus $E$,

$$E = \frac{d\sigma}{d\epsilon} = \kappa \sigma + E_0, \quad (2.26)$$

where $E_0$ is a material constant. The biaxial stress $\sigma$ in the cornea can be determined by the corneal geometries and the IOP via the Laplace’s law,

$$\sigma = \frac{IOP \cdot r}{2t}. \quad (2.27)$$

The corneal tangent modulus $E$ as a function of IOP has been reported in the literature [39, 66] and this data showed that $E$ varies linearly with IOP. Using the indentation method [66], the corneal elastic tangent modulus $E$ for the porcine eyes can be plotted as a function of corneal biaxial stress $\sigma$ in this study (Figure 2-11). The plot shows that $E$ varied with $\sigma$ with $\kappa = 18.0$ and $E_0 \approx 0$ for IOP between 12 to 40 mmHg. Using the constitutive behavior, Eq.(2.7) can be rewritten as

$$b^* = \frac{\kappa \cdot IOP \cdot r \cdot t^2}{2t \cdot a_e (r - t/2) \sqrt{1 - v^2} \delta^e}. \quad (2.28)$$

Substituting Eq.(2.15) and Eq.(2.17) into Eq.(2.28) and taking $r >> t$, we obtain the functional form for the ratio between the specific pressure force $A^* \cdot IOP$ and the specific corneal resistance force $b^*$,
\[
\frac{A^* \cdot \text{IOP}}{b^*} = \frac{\pi \sqrt{2 - 2\nu^2} D_{GAT} \sqrt{r\delta^\gamma} \frac{dF}{d\delta}}{\kappa_s GAT} \left| a_{ic}\right|.
\]

By taking the R.H.S. of Eq.(2.29) as \( \gamma \), Eq.(2.19) can be obtained. It was found from this study that \( \gamma \) was a constant across the porcine eyes (Figure 2-4).
Figure 2-1. Dependence of corneal tangent modulus $E$ on IOP. Different lines represent different eyes.
Figure 2-2. Experimental setup on a porcine eye.
Figure 2-3. Typical load-displacement data acquired from the indentation on a pressurized porcine eye.
Figure 2-4. The specific pressure force $A^* \cdot IOP$ as a function of the specific corneal resistance force $b^*$ for the porcine eyes ($n=12$, $R^2=0.976$).
Figure 2-5. $IOP_{IST}$ and $IOP_{GAT}$ as a function of $IOP_T$ for the porcine eyes (n=12).
Figure 2-6. Bland-Altman plots showing the difference between $IOP_{IST}$ and $IOP_T$ against $IOP_T$. 
Figure 2-7. Error of $IOP_{IST}$ and $IOP_{GAT}$ as a function of corneal stiffness for the porcine eyes (n=12).
Figure 2-8. The variation of the specific contact area $A^*$ as a function of the corneal stiffness for human eyes with $r=7.8$mm and $t=0.52$mm. The square marker indicates the corneal stiffness assumed by GAT.
Figure 2-9. Error of GAT IOP reading as a function of the corneal stiffness for human eyes with true IOP=15mmHg, $r=7.8mm$ and $t=0.52mm$. The square marker indicates the corneal stiffness assumed by GAT.
Figure 2-10. Schematic diagram of the non-linear stress-strain behavior of the cornea.
Figure 2-11. The corneal tangent modulus $E$ as a function of corneal biaxial stress $\sigma$ for the porcine eyes ($n=12$, $R^2=0.90$).
Chapter 3

3 Effect of age-stiffening and intraocular pressure on optic nerve damage

Abstract

Age-stiffening of ocular tissues is statistically linked to glaucoma in the elderly. In this study, the effects of age-stiffening on the lamina cribrosa, the primary site of glaucomatous nerve damage, were modeled using computational finite element analysis. We showed that glaucomatous nerve damage and peripheral vision loss behavior can be phenomenologically modeled by shear-based damage criterion. Using this damage criterion, the potential vision loss for 30 year old patient with mild hypertension of 25 mmHg intraocular pressure (IOP) was estimated to be 4%. When the IOP was elevated to 35 mmHg, the potential vision loss rose to 45%; and age-stiffening from 30 to 60 years old increased the potential vision loss to 52%. These results showed that while IOP plays a central role in glaucomatous damage, age-stiffening facilitates glaucomatous damage and may be the principal factor that results in a higher rate of glaucoma in the elderly than in the general population. Individual adjustment of the threshold IOP in tonometry based on the information of ocular tissue biomechanical properties may help improve glaucoma risk assessment and disease management for the elderly or people with inherently stiffened eyes.

3.1 Introduction

Optic neuropathy in glaucoma causes visual field loss and blindness [6, 7]. The optic nerve damage in the lamina cribrosa (LC) of the sclera, the primary site of glaucoma, is correlated with the intraocular pressure (IOP) [3, 26, 27, 71]. The linkage between IOP and glaucoma has been investigated using computational models [28-31, 72]. In general, the tangential tensile strains are correlated with IOP. Eyes with high scleral stiffness were predicted to have lower
strains at the LC and the optic nerve head (ONH) [28]. However, these computational results were at odds with the clinical observation that age-stiffened eyes in the elderly [33, 73-75] suffer more nerve damage, not less [76]. Moreover, experimental studies showed that the optic nerves are sheared at high IOP [3]. Since glaucomatous vision loss starts from the periphery, a phenomenologically consistent model should also show that damage starts at the periphery. In this study, we show that the damage progression can be modeled using a shear-based approach.

After the establishment of the phenomenologically consistency, the relation between the ocular stiffness and shear stresses in the LC was quantitatively studied. The finite element analysis shows that the tissue stiffening is an important contributing factor in progressive vision loss. To improve diagnostic accuracy, the ocular tissue stiffness of individual patients should be taken into consideration. A threshold IOP adjustment as a function of ocular tissue stiffness and age is proposed in this study.

3.2 Methods

A 3-dimensional human eyeball model (Figure 3-1) was built in a computer-aided design (CAD) software (Solidworks 2007, Dassault Systemes Solidworks Corp.), and imported to a finite element analysis (FEA) software (ANSYS Simulation 11.0, SP1, ANSYS, Inc.) for computational simulation. The core structural dimensions (Figure 3-1) of the globe and ONH, such as the internal radius of eyeball shell, the scleral thickness and the LC thickness were adapted from Sigal’s study [28]. The eye with adipose tissue was assumed to be axisymmetric about the central axis of the LC. The adipose tissue was set to cover 140 degrees of the eye, and the thickness of the adipose tissue was set to 4.6mm. The LC anterior surface central deflection (LCCD) was assumed to be 0.1mm. The central corneal thickness, corneal diameter and radius of curvature of the cornea were 0.5mm, 11mm and 7.8mm, respectively. The disc diameter was set to be 1.8mm, and a cup-to-disc diameter ratio (CDR) [77] of 0.45 was used.
The shape and the dimensions of the pre-laminar neural tissue were adapted from Sigal [28] and are shown in Table 3-1. Since the blind spot is ~15 degrees nasally from the fovea [9], the 165° angle between the central axis of cornea and that of the ONH region was used. Since variation in the coverage angle of the pre-laminar neural tissue does not significantly affect the deformation within the ONH region, the coverage angle of the pre-laminar neural tissue was set at 80°.

The baseline material properties used in our model are shown in Table 3-1. The baseline elastic moduli of the sclera, LC, retina, optic nerve and diameter were the same as those used by Sigal’s group [28], and the elastic modulus of the cornea was adapted from the paper by Hamilton [41]. The elastic modulus of the adipose tissue attached to the globe was approximated to be the same as that of the soft tissue in human buttocks [78-80]. To examine the effects of tissue stiffness, the scleral elastic modulus, $E_s$, was varied from 1 to 9 MPa and the elastic modulus of the lamina cribrosa, $E_{LC}$, was varied from 0.1 to 0.9 MPa [28].

The intraocular pressure exerted on the inner surface of the pre-laminar neural tissue, the sclera and the cornea by the vitreous body of the eye was simulated by applying normal pressure loads evenly onto the inner surfaces of the eye. The outer surface of the adipose tissues was fixed as the boundary condition. Coarse meshing of the structure was auto-generated by the FEA software. The mesh in the ONH region was manually refined until the output has <0.5% differences even when the mesh density was doubled (Figure 3-2). The numerical accuracy was comparable to the Sigal’s study [28].

### 3.3 Results

#### 3.3.1 Results from finite element analysis

Glaucomatous vision loss starts at the periphery and progresses toward the center [8, 9]. Since the damaged nerves are shown to be sheared [3, 81], shear stresses from the
computational model should be higher at the periphery and lower at the center of the LC. The local maximum shear stresses in the LC from our baseline model at 25mmHg are shown in Figure 3-3. The results showed that the local maximum shear stresses were highest at the peripheral anterior surface and lowest in the central anterior surface. The effect of tissue stiffness on the shear stresses is plotted in Figure 3-4 and Figure 3-5. The data showed that LC stiffening (Figure 3-5) increased the stresses in the LC, while scleral stiffening (Figure 3-4) lowered the shear stresses. When both tissues were stiffened together (Figure 3-6), the shear stresses increased.

The overall average stress behavior in the tissue is shown in Figure 3-7. When both the sclera and the LC were stiffened together (solid triangles), the average shear stress in the LC increased. Since the LC and the sclera stiffen with age [33, 34], aging would increase the shear stresses in the LC even when the IOP is unchanged.

3.3.2 Projected effect of aging and tissue stiffening on glaucoma

Glaucomatous vision loss is positively correlated with IOP and age. The elastic properties of the ocular tissues are also linearly correlated with IOP [34, 75] (Figure 3-8) and nonlinearly correlated with age (Figure 3-9 and Figure 3-10) [33, 73-75]. On the basis of the plots, $E_{LC}$ and $E_s$ for the LC and the sclera at different ages and IOPs were interpolated from the plots and used in the model.

For vision loss, the classical Tresca shear failure criterion (maximum shear stress failure criterion) [82-85] was adopted. Vision was classified as lost when $\tau_a \geq \tau_c$, i.e., when $\tau_a$, the shear stress in a nerve fiber along the thickness direction, exceeded $\tau_c$, the Tresca critical damage shear stress.

$\tau_{\text{max}}$, the maximum shear stresses along the thickness direction in the LC were determined from the model. Statistically, damage is negligible in normal eyes. To satisfy this boundary
condition, $\tau_c$ was taken as 0.0035MPa in the simulations such that $\tau_{max}$ in normal eyes ($E_s=1$MPa and $E_{LC}=0.1$MPa) under mild ocular hypertension (IOP = 25mmHg) does not exceed $\tau_c$. Using the criterion, the damage for normal eye was estimated and is shown in Figure 3-11. The results showed that nerve damage increased with tissue stiffening. Using the correlations established in Figure 3-9 and Figure 3-10, the damage as a function of age is plotted in Figure 3-12. The plot showed the people with IOP less than 25mmHg have less than 30% damage, and normal age-stiffening in the elderly resulted only in minor vision loss.

Since the majority of the general population do not have optic nerve damage and glaucoma [10], the simulation results are in line with population trend.

On the other hand, Figure 3-13 showed that people with inherently higher ocular tissue stiffness suffered 25% to >135% more damage depending on IOP. This means that the elderly with age-stiffened ocular tissues are more susceptible to IOP-induced nerve damage compared to younger people.

### 3.3.3 Effect of aging and tissue stiffening on the threshold IOP

To examine the susceptibility against optic nerve damage for different eyes, Figure 3-11 and Figure 3-12 were re-plotted. By defining the threshold IOP as the IOP where the optic nerve started to suffer damage, the effect of ocular tissue stiffness on the threshold IOP relative to $E_s=1$MPa (Figure 3-14) and the effect of aging on the threshold IOP relative to age=30 (Figure 3-15) were plotted. The threshold IOP can be up to 7 to 10 mmHg lower in stiffer eyes than in more compliant eyes. The relation can be described by the following empirical fittings:

$$IOP_{threshold, \ relative \ to \ E_s=1MPa} = -11.1 + 31.6 \cdot \exp(-1.0 \cdot E_s), \ R^2 = 0.99$$  \hspace{1cm} (3.1)

$$IOP_{threshold, \ relative \ to \ age=30} = -0.00116 \cdot age^2 - 0.010 \cdot age - 1.3, \ R^2 = 0.99$$  \hspace{1cm} (3.2)
3.4 Discussion

The shear stress-based model successfully explained the general observation that glaucomatous vision loss often starts from the periphery. When used together with the Tresca failure criterion, we showed that age-stiffened eyes are more sensitive to nerve damage from elevated IOP. The results from this study showed that the nerve damage from IOP is amplified by high ocular tissue stiffness, but high ocular stiffness itself does not cause nerve damage. The results also showed that age-stiffened eye are undamaged if IOP is normal. This is in line with the observed trend that the majority of the elderly who have normal IOP do not suffer from glaucoma. For the population of people with age-stiffened eyes, the results showed that they are more sensitive to nerve damage from elevated IOP. These are people with higher risk and should be monitored more frequently than people with normal ocular stiffness.

To identify the people at higher risk for glaucoma, methods for characterizing the in vivo ocular tissue stiffness should be developed. Individual adjustment on the threshold IOP (21 mmHg) based on information regarding ocular tissue biomechanical properties and age (Figure 3-14 and Figure 3-15) may improve the accuracy of glaucoma risk assessment. This new risk assessment criterion will have to be examined in clinics for revising the model with clinical data.

3.5 Remarks

The foregoing analysis was developed on the basis of the results from the simplified eye model used in this study. In real eyes, the intraocular pressure is not a constant, but varies in a 24-hour cycle and the properties are generally viscoelastic and its behaviors are time-dependent. In most computational models of the eye, these complex features were generally ignored because their inclusion requires further assumptions about the viscoelastic material laws and
material parameters that govern them. With an already long list of parameters in the model, the inclusion of more parameters may not lead to a better understanding of the eye behavior, but more confusion. By recognizing the pitfalls of complex models, we have chosen to add only two essential elements into the shear-focused model, i.e., age-dependence and damage criterion, while following the linear elastic modeling approach used in the literature [28-31, 72]. Both elements were added with reference to the real behaviors and checked against the clinical trends. The age-dependence of the sclera was developed from experimental data. Comparison showed that the relation is in good agreement with reported data. The second element added was the nerve damage criterion. Since the experimental evidence indicated that the nerves were damaged by shear [3, 81], the classical criterion for shear stress, the single parameter Tresca shear failure criterion was borrowed from classical failure mechanics [82-85] and adopted as the initial criterion for nerve damage. A crucial test of the criterion is not only whether the criterion predicted nerve damage at high IOP, but minimal damage when IOP is low. Computational results in this study showed that the Tresca criterion reasonably predicted the behavior. More complex damage models that account for potential dependence on age, anisotropic behavior of nerve fibers and time dependence can be added, but as a first criterion, the Tresca criterion appears to be sufficient since it modeled observed behavior. Refinement can be incorporated when clinical data on age-dependence are available.

3.6 Conclusions

Optic nerve damage from high IOP can be reasonably characterized by shear-based Tresca criterion. The study showed that shear stresses in the LC are higher in eyes with stiffened ocular tissues subjected to the same IOP. Consequently, in addition to IOP, the ocular stiffness of the eye should also be measured in vivo to improve risk assessment accuracy and to identify high-risk glaucoma patients for alternate aggressive treatment to arrest vision loss. A
new threshold IOP criterion based on individual ocular biomechanical properties is proposed and will be tested in clinics.
Figure 3-1. FEM model used in this study. The dimensions and material properties of tissues are detailed in Methods and in Table 3-1.
Figure 3-2. Mesh of the FEM model.
Figure 3-3. Effect of elevated IOP on shear stresses in the LC: Shear stress distribution in the diametrical cross-section of the LC for the case of IOP=25mmHg, $E_s=3\text{MPa}$ and $E_{LC}=0.3\text{MPa}$ is shown. The radial position is as defined in Figure 3-1.

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Figure 3-13. Effect of aging on nerve damage in inherently stiffened eyes: Percentage nerve damaged (for \( \tau_c = 0.0035\text{MPa} \)) against IOP at different ages for tripled \( E_s \) and \( E_{LC} \) of that in Figure 3-9 and Figure 3-10.
Figure 3-14. Effect of ocular tissue stiffness on threshold IOP (relative to $E_s=1$ MPa).

Figure 3-15. Effect of aging on threshold IOP (relative to age=30).
Table

Table 3-1. Summary of the geometric parameters, material properties and mechanical loading in the FEM eyeball model used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Unit</th>
<th>Baseline value</th>
<th>Sources/ References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal radius of the globe</td>
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<td>12.0</td>
<td>[28]</td>
</tr>
<tr>
<td>Scleral thickness of the globe</td>
<td>mm</td>
<td>0.8</td>
<td>[28]</td>
</tr>
<tr>
<td>Scleral thickness close to LC</td>
<td>mm</td>
<td>0.4</td>
<td>[28]</td>
</tr>
<tr>
<td>LC central thickness</td>
<td>mm</td>
<td>0.3</td>
<td>[28]</td>
</tr>
<tr>
<td>Retinal thickness</td>
<td>mm</td>
<td>0.2</td>
<td>[28]</td>
</tr>
<tr>
<td>LC anterior surface diameter</td>
<td>mm</td>
<td>1.9</td>
<td>[28]</td>
</tr>
<tr>
<td>Central corneal thickness</td>
<td>mm</td>
<td>0.5</td>
<td>[86]</td>
</tr>
<tr>
<td>Corneal diameter</td>
<td>mm</td>
<td>11</td>
<td>[86]</td>
</tr>
<tr>
<td>Corneal radius of curvature</td>
<td>mm</td>
<td>7.8</td>
<td>[38, 87]</td>
</tr>
<tr>
<td>Pia mater thickness</td>
<td>mm</td>
<td>0.06</td>
<td>[28]</td>
</tr>
<tr>
<td>LCCD</td>
<td>mm</td>
<td>0.10</td>
<td>[28]</td>
</tr>
<tr>
<td>Canal wall angle to the horizontal</td>
<td>deg</td>
<td>60</td>
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<tr>
<td>Optic nerve angle</td>
<td>deg</td>
<td>80</td>
<td>[28]</td>
</tr>
<tr>
<td>CDR</td>
<td>—</td>
<td>0.45</td>
<td>[88]</td>
</tr>
<tr>
<td>Cup depth</td>
<td>mm</td>
<td>0.33</td>
<td>[28]</td>
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<tr>
<td>Peripapillary rim height</td>
<td>mm</td>
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</tr>
<tr>
<td>IOP</td>
<td>mmHg</td>
<td>25</td>
<td>[28]</td>
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<tr>
<td>Poisson ratio of all material</td>
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<td>0.49</td>
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<tr>
<td>Elastic modulus of adipose tissue</td>
<td>MPa</td>
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<td>Elastic modulus of cornea</td>
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<td>[28]</td>
</tr>
<tr>
<td>Elastic modulus of pre-laminar neural tissue</td>
<td>MPa</td>
<td>0.03</td>
<td>[28]</td>
</tr>
<tr>
<td>Elastic modulus of post-laminar neural tissue</td>
<td>MPa</td>
<td>0.03</td>
<td>[28]</td>
</tr>
</tbody>
</table>
Chapter 4

4 Non-invasive measurement of scleral stiffness and tangent modulus

Abstract

Although the sclera has an important role in glaucoma, there is as yet no available method for \textit{in vivo} measurement of scleral biomechanical properties. Therefore an instrumented non-invasive indentation technique for measuring the scleral stiffness and the scleral tangent modulus is developed in this study. The scleral load-displacement responses were measured with a universal testing machine (MTS Alliance RT/5, USA) as a function of intraocular pressure in 15 porcine eyes \textit{ex vivo} using a 5-mm-diameter cylindrical flat-punch indenter. The scleral radius of curvature and scleral thickness were measured using a DSLR camera (Alpha 900, Sony) and a camera-mounted stereomicroscope (M205C, Leica Mircosystems, Wetzlar, Germany), respectively. The relationships between scleral stiffness, tangent modulus and intraocular pressure were examined. The mean local scleral radius of curvature and scleral thickness were 7.86 ±0.49mm and 1.03 ±0.14 mm, respectively. The mean scleral stiffness and scleral tangent modulus of porcine eyes were 0.13 ±0.02 N/mm and 0.20 ±0.04 MPa at 15 mmHg, respectively. Both the scleral stiffness and scleral tangent modulus were positively correlated with intraocular pressure (scleral stiffness: 0.989<r<0.999, p<0.001; scleral tangent modulus: 0.989<r<0.999, p<0.001). From this study, the scleral indentation technique can provide a non-invasive approach to measure scleral stiffness and tangent modulus.

4.1 Introduction

The sclera constitutes over 70% of the outer envelope of the eyeball, has a complex multilayered structure and plays a central role in providing structural stability to the eye. The scleral biomechanical property is an important parameter characterizing the ocular structural
integrity. The sclera has been shown to be more flexible and less load bearing in myopes than in emmetropes [89-93]. It has also been shown that increased ocular rigidity (a measure describing the relationship between the change in intraocular pressure (IOP) and the change in eyeball volume) is associated with the development of glaucoma [73]; and that scleral stiffness is correlated with increased prevalence of glaucoma and age [33, 76]. While the importance of scleral properties is recognized, in vivo techniques for measurement of the scleral properties are limited. Scleral properties such as scleral stiffness and tangent modulus\(^6\) are ascertained from load-displacement curves of the sclera. The loads are generally imposed onto the eye using inflation methods [75, 94-99] and displacements are ascertained using speckle interferometry [75, 94, 95, 99], digital camera imaging [97, 98] or ultrasound speckle tracking [96]. These inflation-based methods are destructive to the eye, and are unsuited for use in clinical study on human eyes in vivo.

Instead of inflation, surface wave elastometry can also be used to measure the elastic properties of the cornea. In surface wave elastometry [100-102], the ultrasound surface wave propagation time between two points on the cornea is measured, but analysis of the elastic modulus from the propagation time requires an accurate model of the wave propagation-properties of the individual eye.

Alternately, scleral properties can be obtained from indentation [103] load-displacement data using an elastic mechanics model. In indentation, the force is applied through-plane in the direction of the outwardly directed intraocular pressure, and the elastic properties can be delineated using standard elasticity models. Indentation on porcine eyes ex vivo and rabbit eyes in vivo showed that the corneal elastic properties can be obtained using

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\(^6\) Like other biological tissues, the sclera is a complex composite structure with many layers. In investigations of biomechanical properties, the detailed fine structures are often ignored such that the properties measured are interpreted as the material property of the composite structure under a small deformation linear elastic regime.
indentation [103]. In this study, indentation is used to non-invasively measure the scleral stiffness and scleral tangent modulus of the eye.

4.2 Methods

15 enucleated porcine eyes obtained from a local abattoir were examined. The measurements of scleral stiffness and tangent modulus were conducted within 12 hours of the animals being killed and the eyes were kept moist inside an insulated bucket with refrigerants at 4 °C. The extraocular muscles and the extraneous fat were carefully removed before the measurement.

The experimental setup for indentation is shown in Figure 4-1. The enucleated eye was held on a cylindrical test jig. The anterior chamber was cannulated and connected to a manometer and a bottle filled with saline. The IOP was adjusted between 7 mmHg and 39 mmHg by varying the bottle height for each scleral measurement. A needle pressure sensor (OPP-M400, Opsens Inc., Canada) was inserted into the anterior chamber to independently measure the IOP.

In parallel, a 5-mm-diameter cylindrical flat-punch indenter was mounted on a 10-N load cell (MTS 100-090-795, S-Beam type, load resolution: 0.0001 N). The indenter assembly was then screw mounted onto the crosshead of a universal testing machine (UTM, MTS Alliance RT/5, USA). The prepared eye was placed underneath the indenter assembly such that the indenter was positioned approximately 10 mm away from the limbus at 12 o’clock along the superior sclera. After alignment, the UTM moved the crosshead-mounted indenter downward at a rate of 20 mm/min to indent the sclera to a depth of 1 mm. The load (F) - displacement (\(\delta\)) data from the indentation were recorded during the indentation. Figure 4-2 shows the typical load-displacement data from the porcine sclera. The loading portion of the load-displacement data was used to determine the scleral properties. This is because the indentation speed and
the loading conditions in the loading portion of the indentation can be controlled by the testing machine, but the behavior of the porcine eyes in the unloading portion of the indentation (e.g. the recovery of the internal viscous fluid) is uncontrollable.

The indentation load-displacement behavior during loading can be divided into an initial partial contact regime and a second full contact regime when the indentation depth is greater than $\frac{r_0^2}{2R}$ (see Figure 4-3). To simplify analysis, the full contact regime with constant contact area is used for determination of the scleral properties in this study (see Appendix for details). The load-displacement curve in the full contact regime is linear, and the tangent modulus [103], a scleral property that describes the stress-strain curve at a specific stress for the sclera\(^7\), can be used to characterize its elastic behavior. In this study, a scleral tangent modulus, $E|_{IOP}$, defined as the slope of the stress-strain curve at fixed IOP [67],

$$E|_{IOP} = \frac{a(R - t/2)\sqrt{1 - \nu^2}}{t} S|_{IOP}, \quad (4.1)$$

is used to describe the scleral indentation behavior. In Eq.(4.1), $S|_{IOP}$ is the scleral stiffness\(^8\) derived from the slope of the load-displacement curve for an eye held at constant IOP. $a$ is a geometric constant of the indentation, $\nu$ is the Poisson’s ratio of the sclera. The sclera was assumed to be incompressible such that $\nu = 0.5$. The value of $a$ depends on $\mu$ (Table 4-1), which in turns depends on the thickness and radius of curvature of the sclera as shown in Eq.(4.2). (See Appendix for more details).

---

\(^7\) The sclera is a structure consisting of multiple tissue layers. The scleral tangent modulus describes the structural response of the sclera as a whole, and is not the properties of the stroma or individual layers within.

\(^8\) Stiffness is the rigidity of an object and represents the extent to which it resists deformation in response to an applied force. Unlike the geometrically-independent material properties – tangent modulus, stiffness is dependent on the geometry of the object other than the mechanical properties. To measure and calculate the tangent modulus, both the geometry and the slope of the load-displacement curve during the indentation are needed. Thus, the scleral stiffness is defined as the slope of the linear load-displacement curve in the model, which represents the resistance of the sclera in response to the applied force by the indenter.

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Table 4-1. Relation between $\mu$ and $a$ from Young et al [67].

<table>
<thead>
<tr>
<th>$\mu$</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
<th>1.2</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>0.433</td>
<td>0.431</td>
<td>0.425</td>
<td>0.408</td>
<td>0.386</td>
<td>0.362</td>
<td>0.337</td>
<td>0.311</td>
<td>0.286</td>
</tr>
</tbody>
</table>

where $\mu$ is determined by using,

$$
\mu = r_o' \left[ \frac{12(1-\nu^2)}{(R-t/2)^2 t^2} \right]^{1/4},
$$

and $r_o'$ is determined by:

$$
\begin{cases}
    r_o' = \sqrt{1.6r_o^2 + t^2} - 0.675t, & \text{if } r_o < 0.5t \\
    r_o' = r_o, & \text{if } r_o \geq 0.5t
\end{cases}
$$

where $r_o$ is the radius of the cylindrical indenter.

$R$ is an effective radius for ellipsoidal sclera. Following Lazarus et al [104], $R$ is defined as,

$$
R = \frac{R_b^2}{R_a},
$$

where $R_a$ and $R_b$ are the local radii of the sclera underneath the indent before indentation was used in Eq.(4.1) to account for the ellipsoidal geometry. To determine $R_a$ and $R_b$, photos of the tested eyes were taken using a DSLR camera (Alpha 900, Sony). The major radius $R_a$ and minor radius $R_b$ of the sclera were obtained by fitting an ellipse onto the boundary (Figure 4-4(a)) using Matlab (R2013a, The MathWorks, Inc.). $t$ is the scleral thickness at the indentation region, and was measured by a camera-mounted Leica M205C stereomicroscope (Leica Mircosystems, Wetzlar, Germany) after scleral sectioning (Figure 4-4(b)). In clinical practice, sectioning is not used and the thickness is measured non-invasively using optical coherence tomography (OCT), pachymetry, or other ultrasound methods.
The scleral tangent modulus was examined as a function of the in-plane biaxial stress in the sclera. When a spherical shell is pressurized, the in-plane biaxial stress in the membrane $\sigma_s$ is described by Laplace’s Law [97],

$$\sigma_s = \frac{R}{2t} IOP,$$

where $t$ is the thickness and $R$ is the effective radius. The scleral stress in the pressurized sclera is determined using this relation [97].

### 4.3 Results

The mean scleral radius of curvature $R$ and scleral thickness $t$ (n=15) were 7.86 ±0.49mm and 1.03 ±0.14 mm respectively. Figure 4-3 shows a typical load-displacement curve obtained from the scleral indentation on a porcine eye measured ex vivo. The curve was linear and the slope represented the scleral stiffness $S|_{IOP}$.

The scleral tangent modulus $E|_{IOP}$ of the eye was then computed by Eq.(4.1). The $S|_{IOP}$ and $E|_{IOP}$ of all the 15 enucleated porcine eyes were measured and plotted in Figure 4-5 and Figure 4-6 as a function of IOP, respectively. Both plots show that the $S|_{IOP}$ and $E|_{IOP}$ were positively correlated with IOP ($S|_{IOP}$ : 0.989<r<0.999, p<0.001; $E|_{IOP}$ : 0.989<r<0.999, p<0.001). The mean scleral stiffness and scleral tangent modulus were 0.13±0.02 N/mm and 0.20 ±0.04 MPa at 15mmHg, with a range between 0.10 N/mm and 0.16 N/mm and between 0.14 MPa and 0.27 MPa, respectively. The mean scleral stiffness and scleral tangent modulus increased at a rate of 0.0065±0.0010 N/mm per mmHg and 0.011±0.0025 MPa per mmHg with

---

9 The two principal stresses are identical and are defined as $\sigma_s$, for an ellipsoid with a locally spherical pole.
IOP, respectively.

The $E_{IOP}$ as a function of the in-plane biaxial stress $\sigma_s$ is plotted in Figure 4-7. The sclera was a stress-dependent material and $E_{IOP}$ was linearly correlated with $\sigma_s$ ($0.989 < r < 0.999$, $p < 0.001$).

4.4 Discussion

This study shows that scleral stiffness and scleral tangent modulus can be non-invasively determined using an indentation technique. Both the scleral stiffness and the scleral tangent modulus increased linearly with IOP (Figure 4-5 and Figure 4-6). Similar to other biological tissue structure, the sclera is less distensible at a high stress (Figure 4-7). This behavior is in line with the findings from the uniaxial tensile strip tests in which the sclera was shown to have a higher tangent modulus when stressed [62, 105, 106].

The scleral tangent modulus measured in this study ($E_{IOP} = 0.20\text{MPa at 15mmHg}$) was approximately twice that of the corneal tangent modulus measured in our previous study ($E_{IOP} = 0.12\text{MPa at 15mmHg}$) using the same indentation technique [103]. This suggests that the sclera is stiffer than the cornea, providing key structural support to the eye. The scleral tangent moduli of porcine eyes measured in this study are comparable to the results reported by Pierscoioneck using the inflation test (0.2 to 0.5 MPa) (the testing IOP ranged from 15 mmHg to 50 mmHg) [107]. However, they are significantly less than those measured with the tensile test reported by Wollensak and Spoerl (5.95 MPa at 8% strain) [106]. This may be due to the difference in stress borne by the sclera between the studies. In the present study, the IOPs were controlled between 7 to 39 mmHg, and the scleral stress ($\sigma_s$) was found to have varied from 0.003 to 0.03 MPa. The scleral stress reported in the tensile test was 0.25 MPa [106] which was significantly larger than the scleral stress in indentation. Since the tangent modulus varies with
stress, the tangent modulus from tensile tests reported by Wollensak and Spoerl would be higher than ones from indentation.

Nayar et al measured the mechanical properties of porcine sclera using nanoindentation [108]. The tangent modulus was 0.023 MPa (converted from the reduced tangent modulus), which was significantly less than the tangent modulus measured using indentation in this study. Since they used sectioned scleral samples that were not pressurized, the data would be regarded as data from the low stress regime and the modulus would be expected to be lower.

Scleral stiffness describes how the scleral shell behaves when it is subject to loading. For instance, an eyeball with a lower scleral stiffness may potentially be lengthened more than another with a stiffer sclera under the same environment and loading conditions (e.g. same level of IOP). This may be relevant to the development of myopia and glaucoma.

Scleral tangent modulus is a structural property and is independent of the geometry of the sclera (e.g. scleral thickness and radius of curvature). It represents a structural characteristic and describes the mechanical behavior of the sclera with a particular composition and layered structure. When the structure has more crosslinked collagen such as that of the sclera of an older individual, an older person would be expected to have tangent modulus larger than that for the sclera of a younger individual. Computation analysis showed that the scleral tangent modulus increases the stresses in the optic nerve head (ONH) [28, 109], and monitoring of the scleral modulus may potentially be an important parameter in managing ONH stresses and glaucoma.

4.4.1 Limitations and future works

The measurement of the scleral modulus at a specific IOP has been demonstrated, but the act of indentation itself may change the IOP. Indentation needs to be restricted to within a reasonable depth to limit the IOP effect to an acceptable level. In our tests, the indentation depths were limited to 1mm and lower. For indentation depths under 1mm, test data showed
that the IOP change during indentation is less than 3 mmHg/mm. This in turn corresponds to less than a 3% effect on the scleral stiffness and tangent modulus for the porcine eyes in this study (see Appendix for details). Human sclera is expected to behave similarly to the porcine sclera, but tests are needed to determine the indentation effect on human scleral stiffness and tangent modulus before use.

Other than the indentation effect, further studies are needed on indentation locations on human eyes. In clinical practice, the human scleral equator is not easily accessible and scleral indentation may be limited to the area close to the limbus. Further studies on human eyes will be needed to examine the effect of indentation location on scleral mechanical property measurements.

In addition, biological tissues are generally viscoelastic and their biomechanical properties are dependent on the strain rate (Figure 4-8) [110]. The stress level in the tissues of a human eye is relatively stable in most of the time (instead of changing rapidly during indentation). The biomechanical properties of the eye at static condition may therefore be important too. Preliminary results from the porcine eye experiment (n=4) show that the tangent moduli of both the cornea and the sclera measured at static condition were well correlated with the tangent moduli of the corresponding tissues measured by the indentation (Figure 4-9). The ratios between the two tangent moduli measured were universal among different individual porcine eyes (with a ratio of 0.86 for cornea and 0.94 for sclera). The ocular biomechanical properties measured by the indentation may therefore act as an indicator of the ocular biomechanical properties at static condition. Further studies on more porcine eyes and human eyes will be needed to confirm the behavior.

10 The tangent modulus of the ocular tissues at static condition was also calculated from the load-displacement behavior of the ocular tissues, except that the load at each different displacement was measured by holding the displacement until the load stabilized.
To summarize, the scleral stiffness and tangent modulus can be measured non-invasively with an indentation technique. They were positively correlated with both the IOP and the biaxial stress borne by the sclera. *In vivo* measurement of the biomechanical properties of the sclera may provide mechanistic insights into the development of glaucoma and myopia.
Appendix: Indentation Analysis

The Goldmann Applanation Tonometry is derived from the modified Imbert-Fick Law, which is a force balance between the measured applied force $F$, surface tension force of the tear film $s$, pressure force $A \cdot IOP$ and material resistance force $b$ [25],

$$F + s = A \cdot IOP + b$$  \hspace{1cm} (A1)

where $A$ is the applanation contact area. To determine the pressure, $F$ is taken at the applanation area $A$.

In instrumented indentation, the sclera is indented to pass $\delta = \frac{r_0^2}{2R}$, where partial contact transits into full contact, to 1 mm where the indenter is in full contact with the sclera. The change in the contact force as a function of indentation depth $\delta$ can be obtained by differentiating Eq.(A1),

$$\frac{dF}{d\delta} + \frac{ds}{d\delta} = \frac{d}{d\delta} (A \cdot IOP) + \frac{db}{d\delta}.$$  \hspace{1cm} (A2)

The area of scleral contact does not change when the indentation depth $\delta$ is greater than $\frac{r_0^2}{2R}$. Once full contact is reached, the applanation area $A$ becomes constant and is independent of $\delta$. In the full contact regime (denoted with subscript fc), $\frac{dA}{d\delta}|_{fc} = 0$, and the contact perimeter is constant. The change of surface tension with indentation depth is also constant such that $\frac{ds}{d\delta} = 0$ and the load-displacement behavior in the full contact regime is linear (Figure 4-3). As a result, Eq.(A2) can be simplified to,

$$\frac{dF}{d\delta}|_{fc} = A_{fc} \cdot \frac{d}{d\delta} (IOP) + \frac{db}{d\delta}|_{fc}.$$  \hspace{1cm} (A3)

From experiments, $\frac{d}{d\delta} (IOP)$ in the full contact regime ranges from 1 to 3 mmHg/mm. When combined with the indentation full contact area, i.e., $A_{fc} \cdot \frac{d}{d\delta} (IOP)$, the contribution of
this term is at most 3% of \( \frac{dF}{d\delta} \) and can be ignored. Consequently, Eq.(A3) can be simplified to,

\[
\frac{dF}{d\delta} = \frac{db}{d\delta}
\]  

(A4)

where the term on the left is the slope of the load-displacement curve in the full contact regime \( S |_{IOP} \). From Young [67], the scleral resistance force \( b\delta \) can be written as,

\[
\frac{b}{\delta} = \frac{E |_{IOP} \cdot t^2}{a(r - t) / 2 \sqrt{1 - v^2}}.
\]  

(A5)

By combining Eq.(A4) and Eq.(A5), Eq.(4.1) can be obtained.
Figure 4-1. Experimental setup for measuring scleral stiffness and tangent modulus of an enucleated porcine eye. One needle inserted to the anterior chamber of the porcine eye was connected to a manometer for IOP control, while the other one was embedded with a pressure transducer for IOP measurement.
Figure 4-2. Typical loading and unloading load-displacement data acquired during the indentation on the porcine sclera. The upper and lower curves are corresponding to the loading and unloading behaviors, respectively.
Figure 4-3. Typical load-displacement data acquired during the indentation on the porcine sclera. The flat-punch indenter tip is in full contact with the sclera when \( \delta > \frac{R_0^2}{2R} \).
Figure 4-4. Measurement of (a) scleral radius of curvature and (b) scleral thickness at the indentation region.
Figure 4-5. Relation between scleral stiffness and IOP. The lines represent experimental data from different porcine eyes (n=15).
Figure 4-6. Relation between scleral tangent modulus and IOP. The lines represent experimental data from different porcine eyes (n=15).
Figure 4-7. Relation between scleral tangent modulus and in-plane stress in the sclera. The lines represent experimental data from different porcine eyes (n=15).
Figure 4-8. Viscoelastic model for ocular tissues [110].
Figure 4-9. Preliminary results (n=4) of the tangent modulus measured at static condition against the tangent modulus measured by indentation (a) in porcine cornea and (b) in porcine sclera.
Chapter 5

5 Ocular biomechanical properties in human cadaver eyes

Abstract

The indentation technique developed for measuring the ocular biomechanical properties in Chapter 4 was adapted to measure the biomechanical properties in human cadaver eyes in this study. Ten enucleated human cadaver eyes (age: 59 to 82) were obtained from a local university. The eyes were indented by a 2-mm-diameter cylindrical flat-punch indenter using a universal testing machine, and the load-displacement data were used for the analysis of the ocular biomechanical properties. Results showed that the stiffnesses and tangent moduli of both the human cornea and sclera increased linearly with IOP or the stress borne by the corresponding tissues. The mean corneal and scleral stiffnesses were 0.044 ±0.004 N/mm and 0.057 ±0.011 N/mm at 15 mmHg, while the mean corneal and scleral tangent moduli were 0.116 ±0.011 MPa and 0.897 ±0.270 MPa at 15 mmHg. The results showed that the indentation technique can be used to characterize the biomechanical properties of human ocular tissues, and to quantify the stiffening effect of the cross-linking agent on the ocular tissues.

5.1 Introduction

The measurement of ocular biomechanical properties could be useful in the risk assessment of glaucoma and myopia, but as yet a non-invasive method to measure them in clinics has been unavailable. A non-invasive indentation technique for the measurement of scleral biomechanical properties is developed and was demonstrated on ex vivo porcine eyes in Chapter 4. In this Chapter, the method is adapted to measure the in vivo ocular biomechanical properties of human eyes. The correlations between corneal and scleral...
biomechanical properties, IOP and the stress born by the ocular tissues were studied in this study. The results show that the human eyes and porcine eyes exhibited similar biomechanical behaviors in terms of the high dependence of IOP and stress in the tissues. The results are also compared to the *ex vivo* human eye data from the literature and the *in vivo* human eye data measured by the same indentation technique in Chapter 6.

5.2 Methods

The human cadaver eye experiments in this study complied with the Declaration of Helsinki and were approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB) (see Appendix).

10 enucleated human cadaver eyes were obtained from the Department of Anatomy at the University of Hong Kong (HKU). The ages of the donors were between 59 and 82. The times between the death of the donors and the days of the experiments were between 80 and 190 days. The measurement of the corneal and scleral biomechanical properties was conducted within 72 hours after enucleation and the eyes were kept at a low temperature (4 °C) inside a refrigerator. The extraocular muscles and the extraneous fat were carefully removed before the measurement.

The experimental setups for the indentation measurement of the cadaver corneas and sclerae are shown in Figure 5-1. The enucleated eye was held on a cylindrical test jig. The vitreous chamber was cannulated and connected to a manometer and a bottle containing saline for controlling the IOP. Normal saline was used to inflate the eye. A needle pressure sensor (OPP-M400, Opsens Inc., Canada) was separately inserted into the vitreous chamber to measure the IOP. The IOP was adjusted between 5 mmHg and 40 mmHg by varying the bottle height for each measurement.

A 2-mm-diameter cylindrical flat-punch indenter was mounted on a 10-N load cell
(MTS 100-090-795, S-Beam type, load resolution: 0.0001 N), which was screw mounted onto the crosshead of a universal testing machine (UTM, MTS Alliance RT/5, USA). The indenter was positioned at the center of the cornea or the superior sclera approximately 10 mm away from the limbus at 12 o’clock. Once the indenter was aligned at the right location, the indenter moved forward at a rate of 20 mm/min to indent the cornea or sclera to a depth of 1 mm. The load ($F$) - displacement ($\delta$) data from the indentation were recorded during the indentation. The photos of the tested eyes were taken using a DSLR camera (Alpha 900, Sony). The photos taken were imported to a computer, and the radii of curvature of the corneas and sclerae were obtained by fitting an ellipse onto the boundary using Matlab (R2013a, The MathWorks, Inc.). The scleral thickness was measured by a micrometer after sectioning the eye.

In this study, a tangent modulus, $E_{IOP}$, defined to be the slope of the stress-strain curve at fixed IOP [67],

$$E_{IOP} = \frac{a(R-t/2)\sqrt{1-v^2}}{t^2} S_{IOP}$$

(5.1)

is used to describe the indentation behavior. In Eq(5.1), $S_{IOP}$ is the stiffness of a cornea or a sclera derived from the slope of the load-displacement curve for an eye held at constant IOP, $a$ is a geometric constant of the indentation, $v$ is the Poisson’s ratio, $R$ is the local radius of curvature, and $t$ is the thickness at the indentation region. The value of $a$ depends on $\mu$ (Table 5-1), which in turns depends on the thickness and radius of curvature of the cornea or sclera as shown in Eq.(5.2).

<table>
<thead>
<tr>
<th>$\mu$</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
<th>1.2</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>0.433</td>
<td>0.431</td>
<td>0.425</td>
<td>0.408</td>
<td>0.386</td>
<td>0.362</td>
<td>0.337</td>
<td>0.311</td>
<td>0.286</td>
</tr>
</tbody>
</table>

in which $\mu$ is determined by using,
\[
\mu = r'_o \left[ \frac{12(1-v^2)}{(R-t/2)^2t^2} \right]^{1/4}
\]  
(5.2)

where \( r'_o \) is determined by:

\[
\begin{align*}
r'_o &= \sqrt{1.6r_0^2 + t^2} - 0.675t, & \text{if } r_o < 0.5t \\
r'_o &= r_o, & \text{if } r_o \geq 0.5t
\end{align*}
\]  
(5.3)

where \( r_o \) is the radius of the cylindrical flat-punch indenter.

Both the cornea and sclera were assumed to be incompressible (i.e. \( v = 0.5 \)). The corneal tangent modulus and scleral tangent modulus were compared to the in-plane biaxial stress born by the corresponding ocular tissue in this study. When a spherical shell is pressurized, the in-plane biaxial stress in the shell membrane \( \sigma \) is described by the Laplace’s Law,

\[
\sigma = \frac{R}{2t} IOP
\]  
(5.4)

where \( t \) and \( R \) are the thickness and local radius of curvature of the shell. \( \sigma \) in the pressurized cornea and sclera can be determined using this relation.

**Cross-linking on human cornea and sclera**

To demonstrate the ability of the non-invasive indentation technique in measuring the changes in ocular tissue biomechanical properties and to examine the cross-linking effect on the human cornea and sclera, the ocular biomechanical properties of one of the cadaver eyes were measured before and after epi-on cross-linking treatment. The cross-linking was done by immersing the intact cadaver eye into a 4% formaldehyde solution with the IOP fixed at 15 mmHg. The immersion procedure lasted for 60 minutes and the eye was then rinsed with water. The stiffness and tangent modulus of the cornea and the sclera were measured before and after the cross-linking procedure. The stiffening effect of the cross-linking on the cadaver eye was studied.
5.3 Results

The mean corneal and scleral radii of curvature ($R_c$, $R_s$) were $7.83 \pm 0.25$ mm and $12.12 \pm 0.36$ mm respectively. The mean corneal and scleral thicknesses ($t_c$, $t_s$) were $0.96 \pm 0.06$ mm and $0.50 \pm 0.08$ mm respectively. Figure 5-2 shows the typical load-displacement curves obtained from the corneal and scleral indentation on the tested cadaver eyes. Similar to the load-displacement curves acquired by indentation on porcine eyes in Chapter 4, the curves from the human cadaver eyes were linear after the flat-punch indenter had fully contacted the corresponding indented tissue.

Both the corneal stiffness $S_c|_{IOP}$ and scleral stiffness $S_s|_{IOP}$ were found to linearly increase with IOP ($S_c|_{IOP} : 0.9994<r<1$, $p<0.001$; $S_s|_{IOP} : 0.9994<r<1$, $p<0.001$), as shown in Figure 5-3. $S_c|_{IOP}$ and $S_s|_{IOP}$ were compared in Figure 5-4, and $S_s|_{IOP}$ was found be higher than $S_c|_{IOP}$ at any IOP. The mean $S_c|_{IOP}$ and $S_s|_{IOP}$ were $0.044 \pm 0.004$ N/mm and $0.057 \pm 0.011$ N/mm at 15 mmHg, with a range between 0.038 N/mm and 0.051 N/mm and between 0.044 N/mm and 0.073 N/mm, respectively. The $S_c|_{IOP}$ and $S_s|_{IOP}$ increased with IOP at a rate of $0.00232 \pm 0.00015$ N/mm per mmHg and $0.00285 \pm 0.00030$ N/mm per mmHg, respectively.

Both the corneal tangent modulus $E_c|_{IOP}$ and scleral tangent modulus $E_s|_{IOP}$ were found to linearly increase with IOP ($E_c|_{IOP} : 0.9994<r<1$, $p<0.001$; $E_s|_{IOP} : 0.9994<r<1$, $p<0.001$), as shown in Figure 5-5. $E_c|_{IOP}$ and $E_s|_{IOP}$ were compared in Figure 5-6, and $E_s|_{IOP}$ was found be higher than $E_c|_{IOP}$ at any IOP. The mean $E_c|_{IOP}$ and $E_s|_{IOP}$ were $0.116 \pm 0.011$ MPa and $0.897 \pm 0.270$ MPa at 15 mmHg, with a range between 0.106 MPa and
0.143 MPa and between 0.553 MPa and 1.352 MPa, respectively. The $E_c|_{IOP}$ and $E_s|_{IOP}$ increased with IOP at a rate of 0.0062 ± 0.0007 MPa per mmHg and 0.045 ± 0.012 MPa per mmHg, respectively.

Both the corneal tangent modulus $E_c|_{IOP}$ and scleral tangent modulus $E_s|_{IOP}$ were also found to linearly increase with the in-plane stress $\sigma$ borne by the corresponding tissue ($E_c|_{IOP}$: 0.9994<\(r<1\), $p<0.001$; $E_s|_{IOP}$: 0.9994<\(r<1\), $p<0.001$), as shown in Figure 5-7. $E_c|_{IOP}$ and $E_s|_{IOP}$ were compared in Figure 5-8, and $E_s|_{IOP}$ was found to be higher than $E_c|_{IOP}$ at any $\sigma$. The $E_c|_{IOP}$ and $E_s|_{IOP}$ increased with $\sigma$ at a rate of 11.27 ± 0.80 MPa per MPa and 27.4 ± 4.60 MPa per MPa, respectively.

**Cross-linking on human cornea and sclera**

The $S_c|_{IOP}$, $E_c|_{IOP}$, $S_s|_{IOP}$ and $E_s|_{IOP}$ before and after cross-linking the cadaver eye by the 4% formaldehyde solution were plotted with IOP, as shown in Figure 5-9. Both the cornea and the sclera had their stiffness and tangent modulus increased after cross-linking. At 15 mmHg, the cornea and sclera were stiffened by about 117% and 39%, respectively.

**5.4 Discussion**

This study utilized the non-invasive indentation methodology developed on porcine eyes to measure the biomechanical properties of the cornea and the sclera of the human cadaver eyes. Similar to the load-displacement curves acquired by indentation on porcine eyes in Chapter 4, the curves from the human cadaver eyes were linear after the flat-punch indenter had fully contacted the corresponding indented tissue. This means that the indentation method developed on porcine eyes can be adapted to characterize the biomechanical properties of
human eyes.

The human eyes and porcine eyes (Chapter 4) exhibited similar *ex vivo* biomechanical behaviors in terms of the high dependence of IOP and stress borne by the tissues. The stiffnesses and tangent moduli of both the human cornea and sclera were found to increase linearly with IOP. Similar to other biological tissues, the cornea and sclera were found to be less distensible at high stress.

From Figures 5-4, 5-6 and 5-8, both the stiffness and tangent modulus of the human sclera were higher than those of the human cornea. This suggests that the sclera is stiffer than the cornea and provides a more important structural support to the human eye. More studies on the scleral biomechanical properties may potentially be useful in understanding the pathology of glaucoma and myopia.

The stiffening effect of cross-linking on the ocular tissue of a human cadaver eye was also measured by the indentation. The demonstration showed that the indentation technique was able to measure the cross-linking effect of the 4% formaldehyde solution on the human cornea and sclera. In the future, altering the biomechanical properties of the ocular tissues, such as crosslinking the cornea or the sclera, may potentially be one of the new directions for the management for myopia and glaucoma. Corneal collagen cross-linking is also found to be the only promising method in preventing the progress of corneal ectasia (also called “keratectasia”), in which the vision is distorted due to abnormal corneal curvature. Although corneal ectasia is infrequent, it is a serious complication of the growing trend for LASIK surgery. The new generation of LASIK surgery begins to combine the LASIK surgery with the corneal cross-linking treatment for stabilizing the corneal shape after the refractive surgery [111, 112]. The non-invasive indentation technique shown in this study can provide a methodology for clinicians to have feedback in the cross-linking treatments by measuring patients’ ocular biomechanical properties in a non-invasive manner before and after the
treatments.

5.5 Limitations and future works

Though the IOP and stress dependence of the biomechanical properties of the human cornea and the human sclera in this study were similar to the behaviors in porcine eyes (Chapter 4) and the data reported by literature [38, 39, 62, 105, 106], both the corneal and scleral tangent moduli measured in this study were relatively small when compared with the reported ex vivo human data in the literature or the in vivo human data measured by the same indentation technique that will be shown in Chapter 6:

From the literature, the ex vivo corneal tangent modulus measured using inflation test was found to be about 0.2 to 3.5 MPa [38, 39]. The ex vivo scleral tangent modulus by different methods from the literature ranged from 1.8 to 5.5 MPa [29, 33, 69, 113-115]. The in vivo clinical data that will be shown in Chapter 6 showed that the mean in vivo human corneal and scleral tangent moduli were 0.620 ±0.108 MPa and 1.935 ±0.329 MPa, respectively. However, the mean ex vivo $E_c|_{IOP}$ and $E_s|_{IOP}$ found in this human cadaver eye study were 0.116 ±0.011 MPa and 0.897 ±0.270 MPa at normal IOP (15 mmHg), which were a few times smaller than both the in vivo data measured by the same indentation technique and the reported data from the literature. This implies that the human cadaver cornea and sclera measured in this study were relatively soft.

This finding may be due to the limitation in this study that all the acquired donor eyes were tested a few months after the date of the death of the donors. The ocular tissues of the donors may probably have degenerated during the long storage and became weaker in mechanical properties. It may also be seen that the cornea of the tested eyes (thickness: 0.96 ±0.06 mm) were much thicker than normal cornea in living human eyes (mean central corneal thickness in Chinese: ~0.55mm [116, 117]). This was due to the corneal stromal edema during
the storage, in which fluid such as the aqueous humor enters the corneal stroma and causes swelling. The measured ocular biomechanical properties in this study may therefore be different from the \textit{in vivo} study or other \textit{ex vivo} studies on human cadaver eyes with a shorter storage period after death.

The human cadaver eyes in this study may be useful in providing insights in the IOP and stress dependence of the human ocular tissues and demonstrating the cross-linking effect on the ocular tissues. These would be more difficult in a clinical study on human subjects. To study the biomechanical properties of human eyes more precisely in the future, \textit{ex vivo} studies on fresher cadaver eyes and more \textit{in vivo} clinical testing will have to be conducted.
Figure 5-1. Experimental setup for measuring the stiffness and tangent modulus of (a) the cornea and (b) the sclera of an enucleated human eye. One needle inserted to the vitreous chamber of the human eye was connected to a manometer for IOP control, while the other one was embedded with a pressure transducer for IOP measurement.
Figure 5-2. Typical load-displacement data acquired during the indentation on (a) the human cornea and (b) the human sclera. The flat-punch indenter tip is in full contact with the cornea and sclera when \( \delta > \frac{r_0^2}{2R} \), where \( \delta \) is the displacement, \( r_0 \) is the radius of the flat punch indenter, \( R \) is the radius of curvature of the indented ocular tissue. The load-displacement relations were linear after full contact.
Figure 5-3. Relations between (a) corneal stiffness $S_{c|IOP}$ and IOP, and (b) scleral stiffness $S_{s|IOP}$ and IOP. Different lines represent experimental data from different human eyes (n=9).
Figure 5-4. Comparison between corneal stiffness $S_c|_{IOP}$ and scleral stiffness $S_s|_{IOP}$ against IOP.
Figure 5-5. Relations between (a) corneal tangent modulus $E_c|_{IOP}$ and IOP, and (b) scleral tangent modulus $E_s|_{IOP}$ and IOP. Different lines represent experimental data from different human eyes (n=9).
Figure 5-6. Comparison between corneal tangent modulus $E_{c|\text{IOP}}$ and scleral tangent modulus $E_{s|\text{IOP}}$ against IOP.
Figure 5-7. Relations between (a) corneal tangent modulus $E_c|_{\text{IOP}}$ and in-plane stress borne by cornea $\sigma_c$, and (b) scleral tangent modulus $E_s|_{\text{IOP}}$ and in-plane stress borne by sclera $\sigma_s$. Different lines represent experimental data from different human eyes (n=9).
Figure 5-8. Comparison between corneal tangent modulus $E_c|_{IOP}$ and scleral tangent modulus $E_s|_{IOP}$ against the in-plane stress borne by the corresponding tissue $\sigma$. The testing range of IOP was from 5 to 40 mmHg.
Figure 5-9. The effect of cross-linking by 4% formaldehyde solution on (a) corneal stiffness $S_{c|IOP}$, (b) corneal tangent modulus $E_{c|IOP}$, (c) scleral stiffness $S_{s|IOP}$, and (d) scleral tangent modulus $E_{s|IOP}$. 
Appendix – Approval for the human cadaver eye experiment

Prof. Jimmy Lai
Ophthalmology
(Rm.301, Block B, Cyberport 4), HKU
29-Nov-13

Dear Prof. Lai,

IRB Reference Number: UW 13-553

The HKU/HA HKW IRB is authorized by a joint agreement of the University of Hong Kong and Hospital Authority Hong Kong West Cluster to review and monitor clinical research. It serves to ensure that research complies with the Declaration of Helsinki and acts in accordance to ICH GCP guidelines, local regulations and Hospital Authority and the University policies.

I write to inform you that your research application/submission has been approved by an expedited process with details shown below. You are also requested to adhere to the conditions listed.

Protocol title: Calibration of soft contact lens sensor for intraocular pressure measurement using human cadaver eyes

Study site(s): As stated in application form

IRB reviewer: Prof. L K Cheung, Deputy Chairman of the HKU/HA HKW IRB

Documents approved:
- 01. Clinical Research Ethics Review Application Form
- 02. Research Protocol; Version 1-181013

Documents reviewed:
- 03. Short CV of Principal Investigator, dated October 2013

Conditions:
1. Do not deviate from, or make changes to the study protocol without prior written IRB approval, except when it is necessary to eliminate immediate hazards to research subjects or when the change involves only logistical or administrative issues.

2. Report the following to HKU/HA HKW IRB: (i) study protocol or consent document change (use HKU/HA HKW IRB RE001/F), (ii) serious adverse event (use HKU/HA HKW IRB RE001/F), (iii) study progress (use HKU/HA HKW IRB RE001/F), (iv) new information that may be relevant to a subject’s willingness to continue participation in the study.

3. Report study progress to HKU/HA HKW IRB at a 12-monthly interval until study closure.

Yours sincerely,

Mr. Chris Yip
HKU/HA HKW IRB Secretary
IRB Reference Number: UW 13-554

The HKU/HA HKW IRB is authorized by a joint agreement of the University of Hong Kong and Hospital Authority Hong Kong West Cluster to review and monitor clinical research. It serves to ensure that research complies with the Declaration of Helsinki and acts in accordance with ICH GCP guidelines, local regulations and Hospital Authority and the University policies.

I write to inform that your research application/submission has been approved by an expedited process with details shown below. You are also requested to adhere to the conditions listed.

Protocol title: Calibration of intracocular pressure measurement with Goldmann tonometer using cadaver eyes with different corneal thickness

Study site(s): As stated in application form

IRB reviewer: Prof. L K Cheung, Deputy Chairman of the HKU/HA HKW IRB

Document(s) approved:
1. Clinical Research Ethics Review Application Form
2. Research Protocol; Version 1-181013

Document(s) reviewed:
3. Short CV of Principal Investigator, dated October 2013

(Conditions:
1. Do not deviate from, or make changes to the study protocol without prior written IRB approval, except when it is necessary to eliminate immediate hazards to research subjects or when the change involves only logistical or administrative issues.

2. Report the following to HKU/HA HKW IRB: (i) study protocol or consent document change (use "HKU/HA HKW IRB RED01F8"), (ii) serious adverse event (use "HKU/HA HKW IRB RED01F8"), (iii) study progress (use "HKU/HA HKW IRB RED01F8") (iv) new information that may be relevant to a subject's willingness to continue participation in the study.

3. Report study progress to HKU/HA HKW IRB at a 12-monthly interval until study closure.)

Yours sincerely,

Mr. Chris Yip
HKU/HA HKW IRB Secretary
Chapter 6

6 In vivo scleral biomechanical properties in human eyes

Abstract

The non-invasive indentation technique for the measurement of the scleral stiffness and tangent modulus has been adapted for use on human cadaver eyes in Chapter 5. In this Chapter, the technique is demonstrated in vivo on 21 human glaucoma subjects and 14 normal subjects in clinics. The in vivo mean scleral stiffness was 0.211 ± 0.073 N/mm from glaucoma subjects and 0.128 ± 0.022 N/mm from normal subjects. The corneal stiffness was found to be positively correlated with the scleral stiffness in this study (R=0.633, p<10^{-5}). The mean in vivo scleral tangent modulus was estimated to be 3.192 ± 1.109 MPa from glaucoma subjects and 1.935 ± 0.329 MPa from normal subjects, which was of the same order of magnitude as the ex vivo human eye results found by invasive techniques in the literature. Further clinical studies on the in vivo human scleral biomechanical properties using the non-invasive indentation technique may be helpful in the risk assessment and diagnosis of glaucoma.

6.1 Introduction

Ocular biomechanical properties could be useful in the risk assessment of glaucoma and myopia, but a non-invasive method to measure them in living humans is currently unavailable. A non-invasive indentation technique for the measurement of corneal and scleral biomechanical properties is developed and was demonstrated on ex vivo porcine eyes in Chapter 4 and ex vivo human eyes in Chapter 5. In this preliminary clinical study, the method is adapted to measure the in vivo scleral biomechanical properties of human eyes. The correlations between corneal and scleral biomechanical properties and IOP are discussed in this study.
6.2 Methods

6.2.1 Locations and subjects

6.2.1.1 Glaucoma subjects

The in vivo human study was carried out at the Chinese University of Hong Kong Eye Clinic (CUHKEC), which is located at the Hong Kong Eye Hospital (HKEH) and is managed under the Department of Ophthalmology and Visual Sciences at the Chinese University of Hong Kong (CUHK). The study was approved by the clinical research ethics committee of the CUHK, which adheres to the tenets of the Declaration of Helsinki. Informed consent was obtained and signed by the subjects after the purposes and possible consequences of the study had been clearly explained.

In this study, the ocular biomechanical properties of 21 glaucoma subjects (42 eyes in total) were examined. The inclusion criteria were that glaucoma patients had their best corrected visual acuity not worse than 20/40. They should have characteristic optic disc features, including narrowing of the neuroretinal rim and/or retinal nerve fiber layer (RNFL) defects with corresponding visual field (VF) defects. Subjects with a history of retinal disease, intraocular surgery or laser procedures, diabetes mellitus, or neurological disease were excluded.

6.2.1.2 Normal subjects

The in vivo human study was carried out at the Optometry Clinic at the Hong Kong Polytechnic University (PolyU) which is managed under the School of Optometry in PolyU. The study was approved by the Departmental Research Committee (see Appendix), which adheres to the tenets of the Declaration of Helsinki.

In this study, the ocular biomechanical properties of 14 normal subjects (28 eyes in total)
were examined. The mean age of the subjects was 23±1. None of the subjects had any symptoms of glaucoma. Subjects with a history of retinal disease, diabetes mellitus, or neurological disease were excluded.

6.2.2 Device configuration and measurement procedure

The central corneal thicknesses and the corneal radii of curvature of the individual eyes were measured by optical coherence tomography (OCT). Goldmann applanation tonometry (GAT) and dynamic contour tonometry (DCT; Swiss Microtechnology AG, Port, Switzerland) were used to measure glaucoma subjects’ IOP at CUHKEC, while only GAT was used to measure normal subjects’ IOP in the Optometry Clinic at PolyU.

A universal testing machine (UTM) was used in the development of the non-invasive indentation measurement of corneal/scleral mechanical properties on porcine eyes. Since it is not practical to use the large machine for human testing in clinics, a miniaturized indentation device is built for the purpose of measuring the mechanical properties of ocular tissues, as shown in Figure 6-1.

The indentation device is composed of 5 units: the mechanical unit, control unit, data storage unit, display unit and power unit. The mechanical unit consists of a miniature linear motorized actuator (without rotational motion during movement) for providing linear motion, and a load cell with a 2-mm-diameter cylindrical flat-punch indenter installed at the front for acquiring the load response from the cornea and sclera. The control unit contains a printed circuit board with a power regulation module and control module. The data storage unit saves the data for further data analysis. The display unit is a monitor showing the data acquired from the tests immediately. The power unit consists of an 11.1V 1600mAh high performance Li-polymer battery. The appearance of the device is designed in a way similar to GAT with a detachable endplate such that the device can fit into the slit-lamps in clinics.
Prior to the indentation measurement on the cornea and sclera, a drop of topical anesthetic is instilled to the subject's eyes. After aligning the flat punch indenter to be in perpendicular contact with the corneal/scleral surface (Figure 6-2), the measurement is started using a remote control. (In the case of the indentation on the sclera, the indentation is done on the temporal sclera and located approximately 5 mm away from the limbus.) The indenter first moves forwards for 1 mm at a rate of 12 mm/sec, then moves back to the original starting position with the same speed and the measurement is done. During indentation, the load-displacement response from the cornea/sclera is acquired. The corneal/scleral stiffness is defined as the slope of the corresponding load-displacement curve at the full contact regime. By inputting the geometry of the individual eye, the corneal and scleral tangent moduli can be computed, as described in Chapter 5.

6.3 Results

6.3.1 Effect of indenter diameter

When the diameter of the flat-punch indenter is too large, there can be a combination of eye dislocation and the deformation on the ocular tissue during the indentation, since the support behind the eye is not totally rigid. This may affect the mechanical property measurement. The applicability of the 2-mm-diameter cylindrical flat-punch indenter for the indentation measurement was first tested before being used in the clinical study:

Indentation measurement was performed on 4 healthy eyes. Each eye was indented by 4 flat-punch indenters with different diameters (2.0, 3.1, 4.1 and 5.1 mm). It was found that there was slight eye dislocation during indentation by the indenters with the diameters as 4.1 and 5.1 mm, but there was no observable eye dislocation during indentation by the indenters with the diameters as 2.0 and 3.1 mm (Figure 6-3). The tangent modulus as a function of indentation diameter measured on 4 healthy eyes was shown in Figure 6-4. It can be seen that the
measurements with the indenter diameters as 4.1 and 5.1 mm showed more divergent results, while the measurements with the indentation diameters as 2.0 and 3.1 mm showed smaller variations and more comparable results. These imply that the 2-mm-diameter indenter would cause minimal eye dislocation and the results measured would be stable and reproducible. The 2-mm-diameter indenter can therefore be applicable for the following clinical measurement on living human eyes in this study. The local deformation on the cornea can be represented by the displacement of the indenter.

6.3.2 Glaucoma subjects

Two typical load-displacement curves of the indentation on the cornea and sclera are shown in Figure 6-5. Similar to the load-displacement curves acquired by indentation on porcine eyes in Chapter 4 and those acquired by indentation on human cadaver eyes in Chapter 5, the curves from the living human eyes were linear after the flat-punch indenter had made full contact with the ocular tissue.

The mean central corneal thickness and corneal radius of curvature of the glaucoma subjects were 0.544 ±0.035 mm and 7.80 ±0.33 mm, respectively. The mean corneal stiffness and scleral stiffness were found to be 0.110 ±0.026 N/mm and 0.211 ±0.073 N/mm, respectively. The mean corneal tangent modulus of the eyes was 0.867 ±0.220 MPa. Since devices for measuring scleral thickness and scleral radius of curvature were not available in the clinics, they were taken as 0.5 mm [118] and 12 mm [28] for the purpose of scleral tangent modulus estimation. The mean scleral tangent modulus was 3.192 ±1.109 MPa.

The in vivo scleral stiffness measured in this study ranged from 0.094 to 0.421 N/mm, and the distribution is shown in Figure 6-6. The scleral stiffness of the individual left eyes is compared with that of the right eyes, as shown in Figure 6-7. The stiffnesses of the fellow eyes are found to be positively correlated (R=0.606, p<0.01). The in vivo scleral stiffness is
plotted against the IOP measured by GAT and DCT (Figure 6-8), and there is no statistically 
Significant correlation (GAT: R=0.215, p=0.171; DCT: R=0.118, p=0.457). The correlation 
Between the corneal stiffness and the scleral stiffness is shown in Figure 6-9 and the two 
Parameters are found to be positively correlated with each other (R=0.633, p<10^{-5}).

6.3.3 Normal subjects

The mean central corneal thickness and corneal radius of curvature of the subjects were 
0.540 ±0.018 mm and 7.82 ±0.34 mm, respectively. The mean corneal stiffness and scleral 
stiffness in normal eyes were found to be 0.078 ±0.013 N/mm and 0.128 ±0.022 N/mm, 
respectively. The mean corneal tangent modulus and scleral tangent modulus were 0.620 
±0.108 MPa and 1.935 ±0.329 MPa, respectively.

The in vivo scleral stiffness measured in this study ranged from 0.081 to 0.159 N/mm, and 
the distribution is shown in Figure 6-10. The scleral stiffness of the individual left eyes is 
compared with that of the right eyes, as shown in Figure 6-11, and there is no statistically 
significant correlation (R=0.108, p=0.713). The in vivo scleral stiffness is plotted against the 
IOP measured by GAT (Figure 6-12), and there is no statistically significant correlation 
(R=0.090, p=0.648). The correlation between the corneal stiffness and the scleral stiffness is 
shown in Figure 6-13 and the two parameters are found to be positively correlated with each 
other (R=0.348, p<0.05).

6.4 Discussion

The in vivo scleral biomechanical properties of 42 human glaucomatous eyes and 28 
normal eyes without glaucoma were investigated in this study. Similar to the 
load-displacement curves acquired by indentation on porcine eyes in Chapter 4 and those
acquired by indentation on human cadaver eyes in Chapter 5, the curves from the living human eyes were linear after the flat-punch indenter had made full contact with the ocular tissue. This means that the indentation method developed on porcine eyes and human cadaver eyes can be adapted to characterize human eyes in vivo.

There is a variety of scleral stiffness among individual eyes. The mean in vivo human scleral tangent modulus estimated in this study and the ex vivo human scleral moduli found in literature are of the same order of magnitude, as shown in Table 6-1. Both the scleral stiffness and scleral tangent modulus found in this clinical study were a few times higher than those found in the human cadaver eye testing in Chapter 5 using the same indentation approach. This may be due to the fact that the tissues of the cadaver eyes tested in Chapter 5 had been degenerated and softened by a few months of storage after death.

The in vivo corneal stiffness was found to be positively correlated with the in vivo scleral stiffness in this study (Figure 6-9). This may imply that the biomechanical properties of different ocular tissues are potentially correlated with each other. As the cornea and sclera are more accessible than the tissues in the optic nerve head (ONH) region in living humans, the corneal and scleral biomechanical properties may be relevant and useful in projecting the biomechanics of the ONH region in glaucoma.

From the clinical data in this study, the in vivo scleral stiffness is around 2 times that of the corneal stiffness, while the in vivo scleral tangent modulus is around 3 to 4 times that of the corneal tangent modulus. As the stiffer sclera occupies over 70% of the outer envelope of an eye, the biomechanical behaviors of the sclera determine the overall biomechanics of the eyeball. The preliminary data showed that the glaucomatous eyes may have a higher scleral stiffness than normal eyes do, but more studies would be needed to account for the differences in age and IOP of the two groups. Further studies and more clinical data on the scleral biomechanical properties could be important in the risk assessment and diagnosis of glaucoma.
and myopia.

Table 6-1. Summary of ex vivo human scleral tangent moduli from the literature [29].

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Authors</th>
<th>Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile</td>
<td>Friberg and Lace [33]</td>
<td>1.8 – 2.9</td>
</tr>
<tr>
<td>Compression</td>
<td>Battaglioli and Kamm [113]</td>
<td>4.76</td>
</tr>
<tr>
<td>Inflation</td>
<td>Woo et al. [69]</td>
<td>5.5</td>
</tr>
<tr>
<td>Inflation + Computation</td>
<td>Kobayashi et al. [114, 115]</td>
<td>5.5</td>
</tr>
</tbody>
</table>

6.5 Limitation and future works

As shown in Chapters 4 and 5, both the porcine eyes and human cadaver eyes were found to have scleral stiffness increasing with IOP. It was, however, found in this study that the in vivo scleral stiffness did not correlate with IOP very well (Figure 6-8). This may be due to the following limitation in the study:

The IOP of the eyes can be invasively controlled by a manometer in the ex vivo study. The correlation between the ocular biomechanical properties and IOP can be acquired for every porcine eye or human cadaver eye, and the correlation for each eye is very linear. This procedure, however, cannot be used in clinical study. Instead, only one set of ocular biomechanical properties at a single IOP level could be acquired for each subject in this study. The data shown in Figure 6-8 and Figure 6-12 were from different eyes at different IOPs. The correlation between the ocular biomechanical properties and IOP for an individual eye was not known. As there can be individual variations in the mechanical properties even at the same IOP level, the stiffness and IOP across different eyes may not be correlated.

To have a better understanding of the correlation between ocular biomechanical properties and IOP, it is suggested to carry out the measurement on patients during ocular surgery where
a manometer may be used to vary the IOP, or to lengthen the study and repeat the measurement on each patient until he or she has a natural variation in IOP. In this way, the correlation between the ocular biomechanical properties and IOP for each individual eye can be determined. From the data on porcine eyes and human cadaver eyes, a positive correlation would be expected.
Figures

Figure 6-1. Instrumented indentation device developed for clinical measurement of the mechanical properties of the ocular tissues.

Figure 6-2. Close view of indentation measurement of ocular biomechanical properties.
Figure 6-3. (a) Slight eye dislocation observed during indentation by an indenter with diameter as 4.1 and 5.1 mm. (b) No observable eye dislocation during indentation by an indenter with diameter as 2.0 and 3.1 mm.
Figure 6-4. Tangent modulus as a function of indentation diameter in 4 healthy eyes.
Figure 6-5. Typical load-displacement curve of the indentation on (a) the human cornea and (b) the human sclera.
Figure 6-6. Distribution of \textit{in vivo} scleral stiffness on 42 human eyes with glaucoma.

Figure 6-7. Correlation between \textit{in vivo} scleral stiffness of fellow eyes from glaucoma subjects. (R=0.606, p<0.01)
Figure 6-8. Correlation between \textit{in vivo} scleral stiffness and IOP on human eyes with glaucoma. (GAT: R=0.215, p=0.171; DCT: R=0.118, p=0.457)

Figure 6-9. Positive correlation between \textit{in vivo} corneal stiffness and scleral stiffness on human eyes with glaucoma. (R=0.633, p<10^{-5})
Figure 6-10. Distribution of *in vivo* scleral stiffness on 28 human eyes without glaucoma.

Figure 6-11. Correlation between *in vivo* scleral stiffness of fellow eyes from normal subjects. 

(R=0.108, p=0.713)
Figure 6-12. Correlation between *in vivo* scleral stiffness and IOP on human eyes without glaucoma. (R=0.090, p=0.648)

Figure 6-13. Positive correlation between *in vivo* corneal stiffness and scleral stiffness on human eyes without glaucoma. (R=0.348, p<0.05)
Appendix – Approval for the clinical study

To Lam Kwok Cheung Andrew (School of Optometry)
From Do Chi Wai, Chair, Departmental Research Committee
Email chi-wai.do@polyu.edu.hk Date 07-Jul-2014

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 14-Jul-2014 to 30-Sep-2014:

Project Title: Precision of corneal tangent elastic modulus measurement in normal human subjects

Department: School of Optometry

Principal Investigator: Lam Kwok Cheung Andrew

Reference Number: HSEARS20140613002

Please note that you will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In the case of the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Departmental Research Committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

You will receive separate email notification should you be required to obtain fresh approval.

Do Chi Wai

Chair

Departmental Research Committee
Chapter 7

7 Concluding Chapter

7.1 Conclusions

Tonometry is the current primary risk assessment method in glaucoma. The measured IOP in tonometry is usually compared to a general threshold IOP level (21 mmHg) as a reference to determine if the subject is at risk. Both the measured IOP and the threshold IOP, however, ignore the individual variations of the ocular tissue biomechanical properties in human eyes. To improve the accuracy of the measured IOP and the clinical relevance of the threshold IOP, the inclusion of individual biomechanical properties is needed. However, a non-invasive way for characterizing ocular tissue biomechanical properties had been unavailable.

In this thesis, a new instrumented corneal indentation method that can account for the individual corneal biomechanical property difference is presented to measure the IOP. The IOP analysis was tested on porcine eyes ex vivo. The results showed that the IOP measurement errors were more than 50% without individual corneal property correction, but were significantly reduced to less than 10% by incorporating the corneal properties into the IOP measurement. The method will be tested on human eyes in clinics.

From the literature, it is found that the pathology of glaucoma may be closely related to the elasticity of the ocular tissues. From the finite element analysis model in this thesis, the Tresca’s shear failure criterion is found to be a potential explanation for the fact that some eyes are more subject to damage by IOP elevation. The shear stress in the optic nerve head region is found to be directly affected by the ocular mechanical properties. Stiffer ocular tissue may increase the risk for glaucoma and lower the threshold IOP an eye can tolerate. Our approach is therefore to enhance the risk assessment and diagnosis of glaucoma by integrating the use of in vivo ocular mechanical properties measurement with the IOP
measurement. The threshold IOP reference in the risk assessment of glaucoma may have to be adjusted individually for eyes with ocular stiffness deviating from that of the general population.

As non-invasive way to characterize ocular tissue properties has until now been unavailable, an indentation technique is developed for the clinical measurement of corneal and scleral biomechanical properties. The technique was tested on *ex vivo* porcine eyes, human cadaver eyes, and *in vivo* human eyes in clinics. The measured results were comparable to the *ex vivo* results measured by other methodologies in the literature. The preliminary clinical results showed that eyes with glaucoma have stiffer ocular tissues than that of the normal population, but more clinical data are needed before making a precise conclusion. The new method can further be tested in clinics to investigate its applicability in the risk assessment and diagnosis of glaucoma.

### 7.2 Contributions

A new instrumented non-invasive indentation tonometry method that can account for the individual variations in corneal stiffness in the measurement of IOP is developed on porcine eyes in this thesis. After verification in clinics, the new tonometry may improve the accuracy of IOP measurement and assist in glaucoma diagnosis and management, in particular for elderly people with age-stiffened eyes and LASIK patients with surgically thinned corneas.

The computational model in this thesis shows the importance of ocular biomechanical properties in glaucoma progression. The current general threshold IOP reference (21 mmHg) may have to be adjusted for eyes with ocular tissue properties which deviate from that of the general population. The indentation methodology developed in this thesis provides clinicians a non-invasive way to measure the ocular biomechanical properties, and has been demonstrated on *ex vivo* porcine eyes, human cadaver eyes and *in vivo* human eyes in this
thesis. After verification by future clinical studies, the method may help clinicians identify people who are more susceptible to glaucoma more accurately, and help prevent glaucoma or reduce vision loss progression rates.

7.3 Limitations and future works

From the characterization data of the human cadaver corneas in Chapter 5, the cornea of the cadaver eyes we acquired was a few times softer (0.116 MPa vs 0.620 MPa) and much thicker (0.96 mm vs 0.54 mm) than the corneas of the living subjects in the clinical study in Chapter 6. This may be due to the fact that the ocular tissues of the donor eyes may have degenerated during the storage process after death. (The time elapsed from the death of the donors to the days of experiments was between 80 and 190 days.) There was also corneal stromal edema, in which fluid such as the aqueous humor entered the corneal stroma and caused swelling. These factors may have greatly altered the biomechanical properties of the ocular tissues of the cadaver eyes we used. To more precisely study the ocular biomechanical properties (Chapter 5) and the new instrumented indentation tonometry (Chapter 2) on human eyes in the future, ex vivo studies on fresher cadaver eyes and more in vivo clinical testing will have to be conducted.

Likewise, more clinical data will be needed to refine the computational model in Chapter 3. The model provides a theoretical basis to show the importance of ocular biomechanical properties in the pathology of glaucoma, and a new threshold IOP criterion based on individual ocular biomechanical properties and age is proposed for glaucoma risk assessment. Longitudinal clinical studies will have to be done to refine the model and the criterion for clinical applications.
Bibliography


[77] Heidelberg Engineering GmbH. Quantitative Three-Dimensional Imaging of the Posterior Segment with the Heidelberg Retina Tomograph [Online].


Publication list

Journal Papers


Conferences

1. L. Leung, et al., "Effect of age-stiffening of ocular tissues on glaucomatous damage."

2. L. Leung, et al., "Development of Cornea-Specific Tonometry (CST): Porcine eye
study," The HKUST International Conference on Biomedical Engineering – Hong Kong, January 11, 2013.

### Appendix – Abbreviation in this thesis

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACG</td>
<td>Angle-closure glaucoma</td>
</tr>
<tr>
<td>CDR</td>
<td>Cup-to-disc ratio</td>
</tr>
<tr>
<td>CHL</td>
<td>Corneal hydration level</td>
</tr>
<tr>
<td>CUHK</td>
<td>The Chinese University of Hong Kong</td>
</tr>
<tr>
<td>CUHKEC</td>
<td>The Chinese University of Hong Kong Eye Clinic</td>
</tr>
<tr>
<td>DCT</td>
<td>Dynamic contour tonometry</td>
</tr>
<tr>
<td>FEA</td>
<td>Finite element analysis</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical user interface</td>
</tr>
<tr>
<td>HKEH</td>
<td>Hong Kong Eye Hospital</td>
</tr>
<tr>
<td>HKU</td>
<td>The University of Hong Kong</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>GAT</td>
<td>Goldmann applanation tonometry</td>
</tr>
<tr>
<td>LASIK</td>
<td>Laser-assisted in situ keratomileusis</td>
</tr>
<tr>
<td>LC</td>
<td>Lamina cribrosa</td>
</tr>
<tr>
<td>LCCD</td>
<td>Lamina cribrosa anterior surface central deflection</td>
</tr>
<tr>
<td>MD</td>
<td>Mean deviation</td>
</tr>
<tr>
<td>NTG</td>
<td>Normal tension glaucoma</td>
</tr>
<tr>
<td>OAG</td>
<td>Open-angle glaucoma</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>OD</td>
<td>Oculus sinister/ Right eye</td>
</tr>
<tr>
<td>ONH</td>
<td>Optic nerve head</td>
</tr>
<tr>
<td>OS</td>
<td>Oculus dexter/ Left eye</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed circuit board</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>POAG</td>
<td>Primary open-angle glaucoma</td>
</tr>
<tr>
<td>PolyU</td>
<td>The Hong Kong Polytechnic University</td>
</tr>
<tr>
<td>RNFL</td>
<td>Retinal nerve fiber layer</td>
</tr>
<tr>
<td>TM</td>
<td>Trabecular meshwork</td>
</tr>
<tr>
<td>UTM</td>
<td>Universal testing machine</td>
</tr>
<tr>
<td>VF</td>
<td>Visual field</td>
</tr>
<tr>
<td>VFI</td>
<td>Visual field index</td>
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